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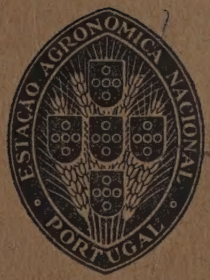

ESTAÇÃO AGRONÓMICA NACIONAL

SACAVÉM

PORTUGAL

3 FEB 1956

FUNGI LUSITANIAE

VII

Auctoribus

MARIA ROSÁLIA DE SOUSA DIAS

ET

EMMANUELE DE SOUSA DA CAMARA

O presente trabalho consta de um género, dez espécies e uma variedade novos e 16 fungos que foram observados no nosso País pela primeira vez.

Aproveitamos o ensejo para agradecer á secção de Botânica da Estação Agronómica o grande obséquio da classificação das plantas invadidas pelos fungos aqui mencionados; muito e muito obrigado, sobretudo ao chefe, engenheiro-agrónomo, sr. PINTO DA SILVA.

UREDINALES (Brongn.) Diet.

PUCCINIACEAE Schröt.

Amerosporae Sacc.

Uromyces Lk.

Uromyces Anthyllidis (Grev.) Schröt. — (*Ured. Lusit.*, I, 410, n. 1).

In foliis vaginisque *Trigonellae Foeni-graeci* L., pr. Cartaxo, leg. D. Amarilis Alberty de Mendonça, aprili, 1953.

Obs.: *uredosporis 20,5-25 × 20-23 μ.; teleutosporis 20-27,5 × × 15-20 μ.*

Uromyces renovatus Syd. — [*Ured. Lusit.*, I, 420 (12), n. 20].

In foliis *Lupini* sp., Torre d'Aires, pr. Luz (Tavira), leg. D. Amarilis Alberty de Mendonça, majo, 1953.

Obs.: *uredosporis 22,5-23,8 × 20-22,5 μ.; teleutosporis 17,5--20,5 × 16-18 μ.*

Uromyces Rumicis (Schum.) Wint. — (*Ured. Lusit.*, I, 421, n. 21).

In foliis caulibusque *Rumicis* sp., pr. Manteigas (Serra da Estrela), leg. António Pereira, novembri, 1953.

Obs.: *Uredosporis 22,5-32 × 20-22,5 μ.; teleutosporis 23,5-27 × × 18,8-22,5 μ.*

Didymosporae Sacc.

Puccinia Pers.

Puccinia Antirrhini Diet. et Holw. — (*Ured. Lusit.*. I, 431, n. 36).

In ramulis *Antirrhini maji* L., in Lisboa (Algés), leg. Alberto Gardé, julio, 1953.

Obs.: *uredosporis 20-23,75 × 19,6-22,5 μ.; teleutosporis 40-65 × × 17-22,5 μ.; pedicellis 45-112,5 × 5-10 μ.*

PYRENIALES (Fr.) Sacc. et Trav.

VALSACEAE Tul.

Allantosporae Sacc.

Valsa Fr.

* 229) **Valsa cenisia** De Not., in Sacc., *Syll.*, I, 110; Ell. et Ev., *Nth. Amer. Pyren.*, 463.

In ramulis *Juniperi phoeniceae* L., pr. Praia do Guincho (Cascais), leg. D. Maria Tereza Lucas, junio, 1953.

Obs.: *ascis 42-45 × 6-7 μ.; sporidiis 9,5-11 × 2,25-2,50 μ.*

** 230) **Valsa ceratophora** Tul., n. var. **minuscula**

Peritheciis 500-750 × 490-670 μ.; collis 650-1000 × 150-160 μ.; ascis clavatis, octosporis, 13-14 × 4,5 μ.; sporidiis subdistichis, allantoideis, hyalinis, 4,5-5,5 × 1,25-2 μ.

Ad ramulos *Hibisci* sp., pr. Vila do Conde (in Stationis Aquicola), leg. D. Aniceta Santos, majo, 1953.

Valsa Pini (Alb. et Schw.) Fr. — (*Mycofl. Lusit.*, VIII et IX, 21, n. 13).

In cortice ramorum *Pini* sp., pr. Pegões, leg. Maria Rosália de Sousa Dias, majo, 1953.

Obs.: *peritheciis 280-380 × 230-310 μ.; rostellis 330-550 × 95-120 μ.; ascis 32,5-39 × 5-6 μ.; sporidiis 5,5-7,5 × 2-2,5 μ.*

Valsae decumbenti (Schmidt.) Nits. affinis.

* 231) **Valsa Saffianoffiana** Sacc. et Berl., in Sacc., *Syll.*, IX, 454.

In ramis *Tamaricis* sp. (an *africanae* Poir.?) pr. Golegã (Ribatejo, leg. Marques de Almeida, decembri, 1953.

Obs.: *peritheciis (cum rostris inclusis) 360-480 × 250-300 μ.; ascis 50-60,5 × 6,25-10 μ.; sporidiis 8,75-11,25 × 2,5-3 μ.*

CERATOSTOMATACEAE Wint.

Hyalodidymae Sacc.

Gnomonia Ces. et De Not.

* 232) **Gnomonia Aceris** Feltg., in Sacc. et D. Sacc., *Syll.*, XVII, 665.

Ad ramulos *Aceris Ginnales* Maxim., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *peritheciis 260-270 × 180-190 μ.; ascis 47,5-55 × 8-10 μ.; sporidiis 10,5-15 × 2,5-4 μ.*

Sociis *Botryosphaeria Berengeriana* De Not. et *Coniothyrio olivaceo* Bon., var. *Aceris* Ferrar.

SPHAERIACEAE (Fr.) Sacc.

Hyalosporae Sacc.

Botryosphaeria Ces. et De Not.

Botryosphaeria Berengeriana De Not. — (*Fg. Lusit.*, I, 103).

Ad ramulos *Aceris Ginnales* Maxim., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *peritheciis 240-260 × 185-240 μ.; ascis 75-80 × 17,5-20 μ.; sporidiis 18,8-26 × 7,5-9,5 μ.*

Sociis *Gnomonia Aceris* Feltg. et *Coniothyrio olivaceo* Bon., var. *Aceris* Ferrar.

Phaeodidymae Sacc.

Didymosphaeria Fck.

Didymosphaeria Rhododendri Oud. — (*Mic. Lusit.*, XI, 124, n. 633).

In ramulis *Rhododendri* sp., pr. Vila do Conde, leg. D. Aniceta Santos, majo, 1953.

Obs.: *peritheciis 340-400 × 230-280 μ.; ascis 100-115 × 7,5-8 μ.; sporidiis 12,5-15 × 5-6,5 μ.*

Hyalophragmiae Sacc.

Calospora Sacc.

* 233) **Calospora arausiaca** (Fabr.) Sacc., *Syll.*, IX, 872; *Pseudo-valsa arausiaca* Fabr., in Sacc., *l. c.*

In ramulis *Quercus* sp., pr. Pegões, leg. Maria Rosália de Sousa Dias, majo, 1953.

Obs.: *ascis 110-120 × 25-27,5 μ.; sporidiis 40-48 × 13,5-13 μ.*

Phaeophragmiae Sacc.

Leptosphaeria Ces. et De Not.

** 234) **Leptosphaeria Aloës** n. sp. (Tab. I, figs. 1-3).

Peritheciis globosis, sparsis vel geminatis, excipulo crasso nigroque, 170-290 × 150-220 μ.; ascis octosporis, cylindraceis, brevissime stipitatis vel nullis, sursum rotundatis, incoloribus, 72,5-85 × 9,75-13 μ.; paraphysibus numerosis, filiformibus, ascis superantibus, achrois; sporidiis subdistichis, cylindraceis, utrinque rotundatis, interdum deorsum plus minusve attenuatis, quinque septatis, rare in initio triseptatis, loculo quarto liniter inflatis, luteolis, 23,75-30 × × 6,25-7 μ.

In foliis *Aloës arborescens* Nill., pr. Sacavém (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, aprili, 1953.

SPHAEROPSIDALES (Lév.) Lind.

SPHAERIOIDACEAE Sacc.

Hyalosporae Sacc.

Cytosporella Sacc.

** 235) **Cytosporella Cercidis** n. sp. — (Tab. II, figs. 1 et 2).

Pycnidiis stromaticis, numerosis, plus minusve ovoideis vel

aliquantum ellipsoideis, nigris, 210-250 × 170-200 μ.; sporophoris filiformibus, fasciculatis, erectis, incoloribus, usque 12 μ. longis; sporulis subellipsoideis vel subcylindraceis, utrinque rotundatis, rectis, biguttulatis (sicut videtur), continuis, hyalinis, 4,5-5,5 × 2,25-2,5 μ.

In ramis *Cercidis Siliquastri* L., pr. Sacavém (ad Hortum Stationis Agronomicae Nationalis, leg. D. Maria Tereza Lucas, julio, 1953.

Socia *Diplodia Siliquastri* West.

Dothiorella Sacc.

** 236) **Dothiorella Macadamiae** n. sp. (Tab. I, figs. 4-5).

Maculis epiphyllis, in apice sitis, cinerescentibus, castaneo cinctis; pycnidiis parum stromaticis, usque 3 aggregatis generaliter, plus minusve rotundatis, excipulo crasso nigroque, 230-300 × 210-250 μ.; sporophoris nullis; sporulis ellipsoideis vel aliquantum ovoideis, rectis, utrinque fere attenuatis, continuis, nubilosis, hyalinis, 17,5-21 × 5-6 μ.

Ad folia *Macadamiae ternifoliae* F. Muell., in Horto Botanico Facultatis Scientiarum, leg. Maria Rosália de Sousa Dias, februario, 1953.

Socia *Hendersonia Macadamiae* n. sp.

** 237) **Dothiorella strobilomorphospora** n. sp. (Tab. I — figs. 6-7).

Pycnidiis cortici innatis, erumpentibus, caespitosis, globosis ellipsoideisve, plus minusve inaequalibus, in apice perforatibus vel quandoque subpapillatis, nigris, 250-400 × 250-280 μ.; sporophoris cylindraceis, fasciculatis, achrois, 5,5-10,5 × 2 μ.; sporulis obclavoideis (strobiliformibus), rectiusculis, utrinque rotundatis, interdum infra parum attenuatis, constinuis, hyalinis, 22,5-26 × 5-6 μ.

In ramulis *Mori nigrae* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, decembri, 1952.

Sociis *Coniothyrio foedans* Sacc. et *Botryodiplodina Mori* n. sp.

Phomopsis Sacc.

* 238) **Phomopsis cryptica** v. Höhn., in Grv., *Sphaeropsid.*, I, 198; *Phoma cryptica* (Nits.) Sacc., *Syll.*, III, 69; Allesch., *Die Pilze*, VI, 221.

Ad ramulos *Lonicerae Caprifolii* L., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 270-280 × 80-110 μ.; sporophoris 1-1,5 μ.; sporulis 7,5-8 × 2,5-3 μ.*

An *Phomopsis Lonicerae* Grv. eadem species erit?

* 239) **Phomopsis Jochromae** Frag., in D. Sacc., Trav. et Trott., *Syll.*, XXV, 136.

Ad ramulos *Jochromae coccinae* Scheid. et *Jochromae tubulosae* Benth., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 190-255 × 120-195 μ.; sporophoris 10-14 × 2 μ.; sporulis 7,5-9,5 × 2-2,5 μ.*

Socia *Diplodia Saccardiana* F. Tassi.

Phomopsis phoenicicola Trav. et Spes. — (*Myc. Lusit.*, XI, 136).

In rachidibus *Phoenicis Loureiri* Kunth., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 210-350 × 70-170 μ.; sporophoris usque 12,5 × × 1,25 μ.; sporulis 8-9 × 2,25-2,5 μ.*

Socia *Microdiplodia Palmarum* Died.

* 240) **Phomopsis Stewartii** Peck., in Sacc. et Trott., *Syll.*, XXII, 901.

Ad ramulos *Cosmi bipinnati* Cav., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. D. Maria Tereza Lucas, februario, 1953.

Obs.: *pycnidiis 150-280 × 100-170 μ.; sporophoris usque 12,5 × × 1,5 μ.; sporulis 7-10 × 2,25-2,5 μ.*

Phyllosticta Pers.

* 241) **Phyllosticta Napi** Sacc., *Syll.*, III, 38; Allesch., *Die Pilze*, VI, 106.

In foliis *Brassicae oleraceae* L., circa Sintra (Almargem do Bispo), februario, 1953.

Obs.: *pycnidiis 65-130 × 60-85 μ.; sporulis 4,5-5,5 × 1,5-2 μ.*

Sphaeronaema Fr.

* 242) **Sphaeronaema Oleae** (De Not.) Sacc., *Syll.*, III, 188; Allesch., *Die Pilze*, VI, 427.

In ramulis *Oleae europaeae* L., pr. Palhoça (Cercal), leg. João Marques de Almeida, majo, 1953.

Obs.: *pycnidiis valde rostratis, 500-600 × 410-430 μ.; sporophoris 17,5-19 μ. longis; sporulis 4,5-6 × 1,5-2 μ.*

Strasseria Bres. et Sacc.

Strasseria Rusci S. Cam. et Luz — (*Myc. Lusit.*, II, 52, n. 95).

Ad folia *Rusci aculeati* L., in Lisboa (Tapada das Necessidades), leg. Maria Rosália de Sousa Dias, novembri, 1952.

Obs.: *pycnidiis 100-130 × 80-125 μ.; sporulis 11-13,8 × 8-8,8 μ.; setulis 4,5-5 × 1,25 μ.*

Phaeosporae Sacc.

Coniothyrium Crd.

* 243) **Coniothyrium foedans** Sacc., *Syll.*, III, 308; Allesch., *Die Pilze*, VII, 41, 43 et 49.

In ramis *Mori nigrae* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, decembri, 1952.

Obs.: *pycnidiis interdum gregariis, 200-300 × 150-210 μ.; sporulis subglobosis uniguttulatisque, elliptici-cylindraceis biguttulatisque, 3-4,5 × 2 μ.*

Sociis *Dothiorella strobilomorphospora* n. sp. et *Botryodiplodina Mori* n. sp.

* 244) **Coniothyrium olivaceum** Bon., var. **Aceris** Ferrar., in Sacc. et D. Sacc., *Syll.*, XVIII, 304.

Ad ramulos *Aceris Ginnales* Maxim., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 300-420 × 280-330 μ.; sporulis 5,5-7,5 × 3-3,75 μ.*

Species pura in Lusitania jam memorata fuit.

Sociis *Gnomonia Aceris* Feltg. et *Botryosphaeria Berengeriana* De Not.

Microhaplosporella S. Cam.

** 245) **Microhaplosporella Brachychitonis** n. sp. — (Tab. II, figs. 3 et 3a).

Pycnidiis stromaticis, monostichis, plus minusve globosis vel irregularibus, fumosis, 170-228×142-170 μ.; sporophoris cylindraceis, erectis, sursum rotundatis, rectis, achrois, 2,5-3×1,5-2 μ.; sporulis rotundatis, ovoideis ellipsoideisve, continuis, eguttulatis, brunneolis, minutis, 5-7,25×4-5 μ.

Ad ramulos *Brachychitonis populnei* R. Br., in Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, aprili, 1953.

Phaeodidymae Sacc.

Botryodiplodia Sacc.

** 246) **Botryodiplodia Brachychitonis** n. sp. (Tab. II, figs. 5 et 6).

Pycnidiis stromaticis, parce locellatis, globosis, excipulo crassiusculo nigerrimoque, 230-330×130-190 μ.; sporophoris cylindraceis, erectis, hyalinis, 9-9,5×3-3,6 μ.; sporulis ellipsoideis subovalibusve, medio uniseptatis, non vel vix constrictulis, utrinque rotundatis, rectis, brunneis, 17-22,5×8-10,5 μ.

In ramulis *Brachychitonis populnei* R. Br., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, aprili, 1953.

** 247) **Botryodiplodia Celtidis** n. sp. (Tab. III, figs. 1-2).

Pycnidiis stromaticis, ellipsoideis, excipulo crassiusculo nigroque, 140-300×80-140 μ.; sporulis plus minusve cylindraceis, rectis, utrinque rotundatis, medio in senilibus uniseptatis, plerumque diu hyalinis demumque atro-brunneis, 19,5-25,5×10-12,5 μ.

Ad ramulos *Celtidis occidentalis* L., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. D. Aniceta Santos, februario, 1953.

** 248) **Botryodiplodia Pistaciae** n. sp. (Tab. III, figs. 5-6).

Pycnidiis epidermide tectis, stromaticis, pluriloculariibus, plus minusve ellipsoideis vel rotundatis, nigris, 200-450×180-260 μ.; sporophoris cylindraceis, numerosissimis, fasciculatis, achrois, minutis,

usque 5,2 μ. longiusculis; sporulis ellipsoideis ovoideisve, utrinque rotundatis, rectis rarissime paulum curvulisve, uniseptatis, non constrictis, brunneis, 17,5-22 × 8-10 μ.

In ramulis *Pistaciae Lentisci* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, augusto, 1953.

** **Botryodiplodina** n. gen.

(*Botryo*, racemus et *Diplodina*, gen.)

Pycnidia botryoso-congesta, stromate basilari suffulta, membranaceo-carbonacea; sporulae ellipsoideo-oblongae, uniseptatae, chlorinae.

** 249) **Botryodiplodina Mori** n. sp. — (Tab. III, figs. 3-4).

Pycnidiis immersis, orbicularibus subglobosisve, pertusis, stromaticis, brunneis, 180-220 × 160-200 μ.; sporulis ellipsoideo-oblongis, uniseptatis (rarissime biseptatis), non constrictis, utrinque lenissime acutatis, plerumque rectis, quandoque aliquantum curvulis, chlorinis, 7-12,5 × 2,5-3,75 μ.

In ramulis *Mori nigrae* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Maria Rosália de Sousa Dias, decembri, 1952.

Sociis *Coniothyrio foedans* Sacc. et *Dothiorella strobilomorphospora* n. sp.

Diplodia Fr.

* 250) **Diplodia Saccardiana** F. Tassi, in Sacc. et P. Syd., *Syll.*, XIV, 932; Allesch., *Die Pilze*, 161, c. icon.; Grv., *Sphaeropsid.*, I, 59.

Ad ramulos *Jochromae tubulosae* Benth., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis sparsis, 225-245 × 140-195 μ.; sporulis 18-23 × × 9-10 μ.*

Socio *Phomopsi Jochromae* Frag.

Diplodia Siliquastri West. — (*Mycofl. Lusit.*, VIII e IX, 61, n. 132).

In ramis *Cercidis Siliquastri* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. D. Maria Tereza Lucas, julio, 1953.

Obs.: *pycnidiis 170-300 × 120-170 μ.: sporulis 20-23,75 × 9,5--10 μ.*

Socia *Cytosporella Cercidis* n. sp.

Microdiplodia Allesch.

Microdiplodia Palmarum Died. — (*Myc. Lusit.*, IX, 67).

Ad folia *Phoenicis Loureiri* Kunth., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 550-710 × 95-120 μ.; sporulis plerumque continuis, rarissime uniseptatis, constrictis, octoformibus, 9-15 × 4,6-6 μ.*

Socia *Phomopsi phoenicicola* Trav. et Spes.

Phaeophragmiae Sacc.

Hendersonia Berk.

Hendersonia Cisti S. Cam., *Myc. Lusit.*, XI, 145, n. 684, c. icon. (Tab. III, figs. 6-8).

Ad ramulos *Halimii (Helianthemi) lasianthi* (Lam.), Grass., pr. Pegões, leg. Maria Rosália de Sousa Dias, majo, 1953.

Obs.: *pycnidiis 240-330 × 140-180 μ.; sporophoris in praesenti observatis, cylindraceis, 15,6-18 × 2,6-4 μ.*

** 251) **Hendersonia Macadamiae** n. sp. (Tab. III, figs. 7-9).

Pycnidiis sparsis, orbicularibus, excipulo crasso nigroque, 115--170 × 110-135 μ.; sporophoris nullis; sporulis plus minusve cylindraceis, quandoque ellipsoideis, utrinque attenuatis, rectis rare curvulisve, triseptatis, non constrictis, chlorinis, 12,5-18,75 × 3-4 μ.

Ad folia *Macadamiae ternifoliae* F. Muell., in Lisboa (ad Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Socia *Dotthiorella Macadamiae* n. sp.

Phaeodictyae Sacc.

Camarosporium Schulz.

* 252) **Camarosporium Orni** P. Henn., in Sacc. et D. Sacc., *Syll.*, XVIII, 372; Grv., *Sphaeropsid.*, II, 96.

Ad ramulos *Fontanesiae phyllireoidis* Labill., in Lisboa (ad

Hortum Botanicum Facultatis Scientiarum), leg. Maria Rosália de Sousa Dias, februario, 1953.

Obs.: *pycnidiis 250-330 × 50-240 μ.; sporulis 13,75-15,5 × × 7-7,5 μ.*

Scolecosporae Sacc.

Septoria Fr.

* 253) **Septoria Agropyri** Ell. et Ev., in Sacc., *Syll.*, XI, 547; *Septor. agrestis* Sacc., in Allesch., *Die Pilze*, VI, 726.

In foliis *Agropyri campestris* Gr. et Godr.. pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Prof. Dr. Branquinho de Oliveira, februario, 1953.

Obs.: *pycnidiis 82,5-99 × 69-80 μ.; sporulis 16,5-35 × 1,5-2 μ.*

* 254) **Septoria Anemones** Desm., in Sacc., *Syll.*, III, 521; Allesch., *Die Pilze*, VI, 730; Grv., *Sphaeropsid.*, I, 368.

In foliis *Anemones palmatae* L., pr. Vila Nogueira (Azeitão), leg. Dr.ª D. Maria de Lourdes de Oliveira, februario, 1953.

Obs.: *pycnidiis 85-100 × 85-90 μ.; sporulis 20-35 × 1,25-2 μ.*

Septoria graminum Desm. — (*Myc. Lusit.*, XII, 209).

In foliis *Tritici vulgaris* L., pr. Sacavem (ad Hortum Stationis Agronomicae Nationalis), leg. Loureiro Ferreira, aprili, 1953.

Obs.: *pycnidiis 105-110 × 95-105 μ.; sporulis 40-54 × 2-2,25 μ.*

HYPHALES (Mart.) Sacc. et Trav.

DEMATIACEAE Fr.

Phaeophragmiae Sacc.

Helminthosporium Lk.

* 255) **Helminthosporium pseudotsugae** B. Cke., *West. Fg.*, II, 251.

In ramulis *Thujae occidentalis* L., pr. Sintra (Tapada do Mouco), leg. D. Aniceta Clotilde dos Santos, julio, 1953.

Obs.: *conidiis delicatis, multiseptatis (8-16); 56,5-88 × 8,75-10 μ.*

An f. *delicata?*

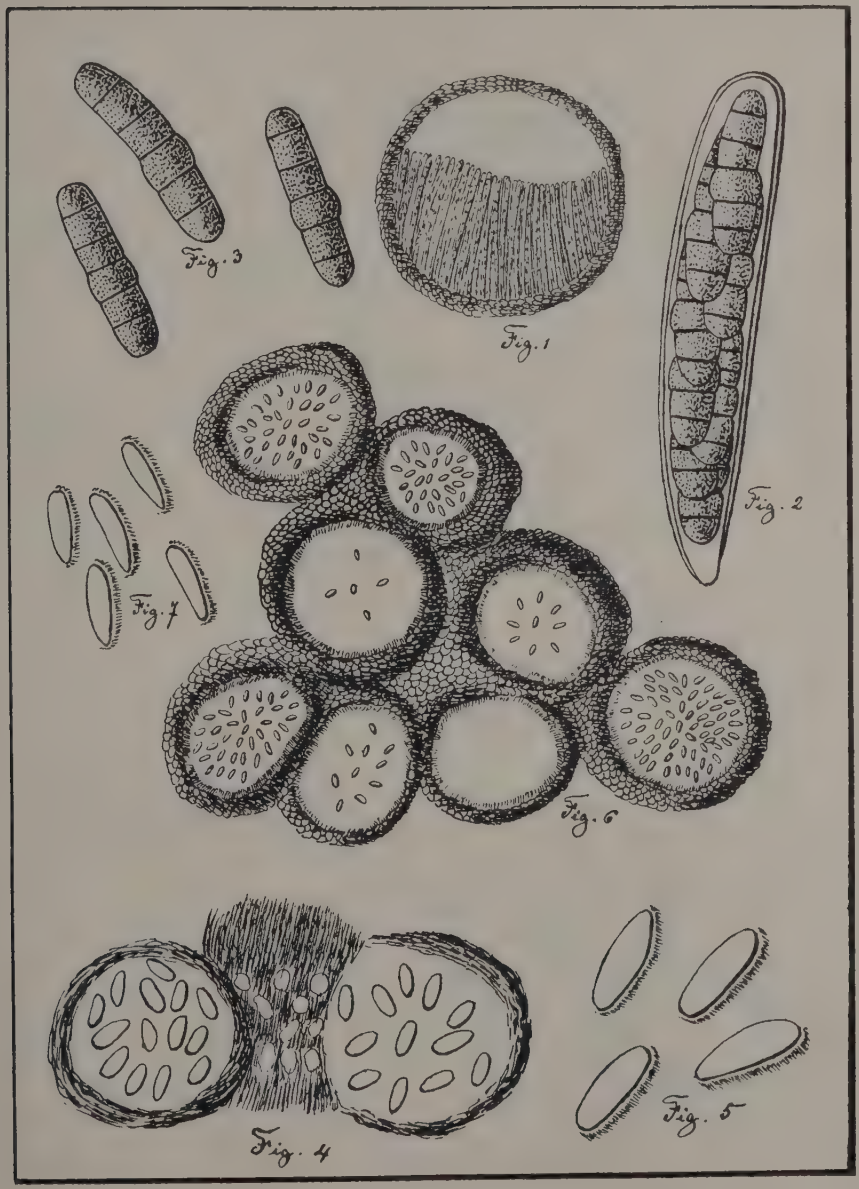
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Fig. 5

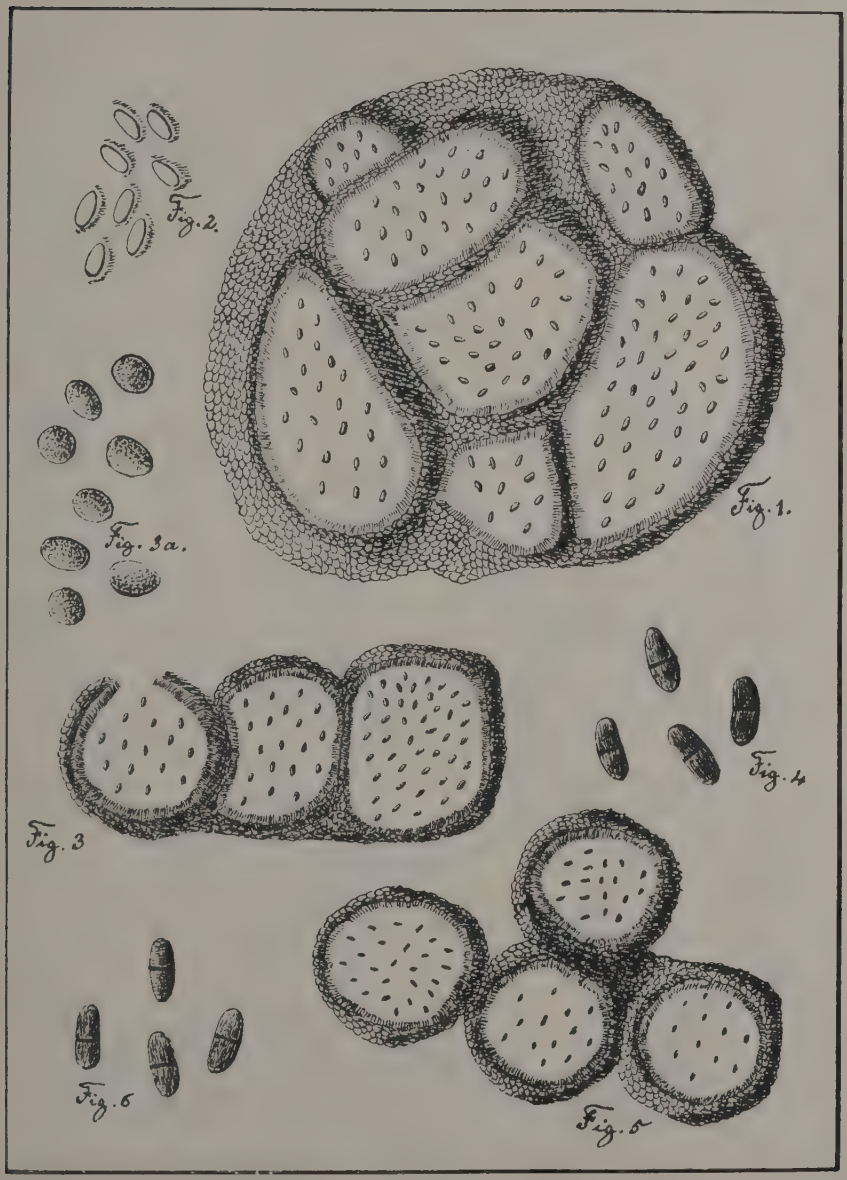
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Fig. 5

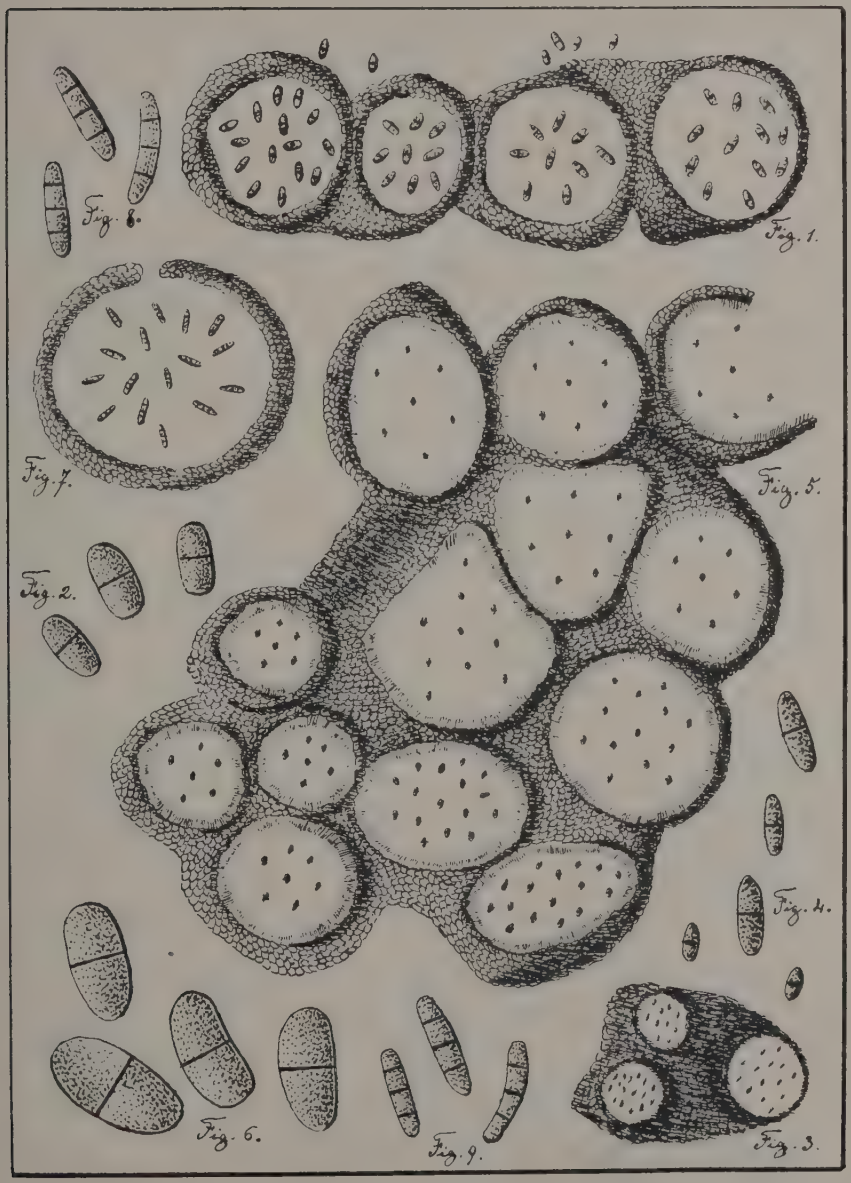
Fig. 8.
Fig. 1.
Fig. 7.
Fig. 5.
Fig. 2.
Fig. 4.
Fig. 6.
Fig. 9.
Fig. 3.

STUDY OF KARYOTYPES AND THEIR ALTERATIONS IN AROIDS

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THE family *Aroidae* is represented in India, specially in Bengal by a large number of genera, species and varieties occurring both in the hills as well as in the plains, reaching up to an altitude of 8,000-9,000 ft. in the Himalayas. The members differ widely as to their preference for habitat, ranging from free floating to aquatic, marshy and terrestrial types. In addition to their occurrence as wides several of the members are also cultivated as ornamental plants and immense horticultural varieties have been raised. It is significant to note that a large number of species, being cultivated as ornamental plants, have become completely sterile, being propagated mainly through vegetative means. It needs no mention that such sterile plants taken together with their fertile relatives form a very ideal material for cytological study. A thorough search into the literature reveals the lack of any critical cytological data on the different members of the family, specially of the Indian ones.

As the species present plants of much horticultural, economic as well as of cytological importance, a critical investigation on the cytogenetics of the different species with the aid of the improved technique was thought highly desirable. Such investigation, it is presumed, would not only throw light on the interpretation of their phylogeny, but also would provide means for their improvement.

The present paper deals with the first part of the investigation where observations on six different species and varieties have been incorporated.

MATERIALS AND METHODS

The following materials were used in the present investigation:

1. *Alocasia fornicata* Scott.
2. *Colocasia antiquorum* Scott. var. *nymphaefolia,* reddish petiole, growing in water.

3. *Gonatanthus sarmentosus* Klotz. var. Petiole black, lower surface of lamina dark-black, upper surface also blackish.
4. *Caladium bicolor* Willd. var. *verschaffeltii*
5. *Caladium bicolor* Willd. var. *chantini*
6. *Caladium bicolor* Willd. (unidentified), spotted variety with black petiole, the lamina being spotted black.

Materials were collected from the suburbs of Calcutta, University Botanic Gardens — Ballygunge, and grown in pots.

After a series of trials in different fixatives, chromic formalin fixation for root tips with increased proportion of formalin, i. e., 1:2 and 1:3 and Nawaschin's fluid for meiotic materials yielded best results. Dehydration and embedding were done as usual and paraffin sections were cut 15 μ-16 μ thick. Newton's crystal violet-iodine technique and premordanting in 1 % chromic acid were found to be essential. Drawings were made with a Leitz microscope using an eyepiece $\times$ 18, 1.3 apochromat objective and an aplanatic condenser $\times$ 1.4 N. A. at a table magnification of $\times$ 3,600 approximately, and for meiotic figures $\times$ 1,800 approximately. The chromosomes of all the species examined sometimes yielded a fuzzy appearance in some of their segments which have been drawn as «dotted» in the figures. These might possibly indicate their heterochromatic nature.

DESCRIPTION

1. *Alocasia fornicata* Scott.

Somatic chromosome number is found to be 28 (Fig. 1). The marked size differences of the chromosomes make them divisible into the following groups (Fig. 1a):

I. Two pairs of long chromosomes.
II. Four pairs of medium-sized chromosomes.
III. Six pairs of short chromosomes.
IV. Two pairs of very short chromosomes.

The size ranges of the chromosomes varies between 2.91 μ to 6.66 μ.

On the basis of their detailed morphology the following types of chromosomes could be classified:

Type A — One pair of long chromosomes with nearly median primary constriction provided with one secondary constriction at the end of the comparatively short arm and two secondary constrictions at the long arm placed equidistant from one another;

Type B — One pair of long chromosomes with nearly median primary constriction, one secondary constriction placed medianly at the short arm and two secondary constrictions at the comparatively long arm;

Type C — One pair of medium-sized chromosomes with nearly median primary constriction and a secondary constriction placed submedian to the long arm;

Type D — One pair of medium-sized chromosomes with nearly median primary constriction and provided with a secondary constriction followed by a long gap at its distal end;

Types E, F — Two pairs of medium-sized chromosomes with nearly submedian primary constrictions and with median secondary constrictions at the long arms. The long arm of the F-pair is, however, comparatively longer than the long arm of the E-pair;

Types G, H — Two pairs of short chromosomes with almost median primary constriction and a secondary constriction located at the middle of the long arm; the latter differs from the former in having the chromatic body in between the primary and secondary constrictions smaller;

Type I — Four pairs of short chromosomes with median primary constrictions;

Type J — Two pairs of very short chromosomes with median primary constrictions.

2. *Colocasia antiquorum* Scott. var. *nymphaefolia*: reddish petiole growing in water.

Somatic chromosome number is 28 (Fig. 2). The chromosomes of this species are shorter than those of *Alocasia fornicata*. In fact, the longest chromosomes correspond to the short chromosomes of *A. fornicata*.

Due to size differences in the set the chromosomes may be divided into two groups (Fig. 2a):

I. Three pairs of long chromosomes (corresponding to short chromosomes of *A. fornicata*).
II. Eleven pairs of short chromosomes.

The size difference of chromosomes is found to vary from 2.77 μ to 6.66 μ.

The following types may be seen:

Type A — One pair of long chromosomes with nearly median primary constriction and with a secondary constriction at the distal end of the long arm;

Type B — In this long pair the secondary constriction is placed very near the primary one towards the comparatively long arm;

Type C — This pair differs from 'B' in that the secondary constriction is placed medianly at the comparatively long arm.

The appearance of these three pairs of secondary constrictions are alike in gross nature and merge one to the other.

Type D — Eleven pairs of short chromosomes with median primary constrictions.

3. *Gonatanthus sarmentosus* Klotz. Petiole black, lower surface of the lamina dark black and upper surface also blackish.

The chromosome-size resembles closely that of *A. fornicata* though they seem to be a bit swollen.

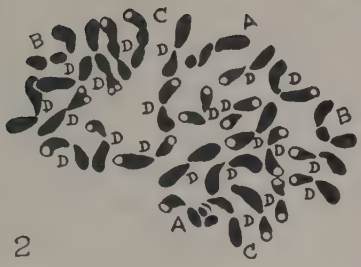
Somatic chromosome number is 28 (Fig. 3). The chromosomes may be classified into the following groups (Fig. 3a):

I. Three pairs of long chromosomes.
II. Nine pairs of medium-sized chromosomes.
III. Two pairs of short chromosomes.

The size difference of chromosome is found to vary between 2.77 μ to 6.66 μ.

PLATE I — Figs. 1-4 - Somatic metaphase plates of *Alocasia fornicata, Colocasia antiquorum* var. *nymphaefolia, Gonatanthus sarmentosus* and *Caladium bicolor* var. *verschaffeltii* respectively. Figs. 1a-4a - Idiograms of the same species.

1



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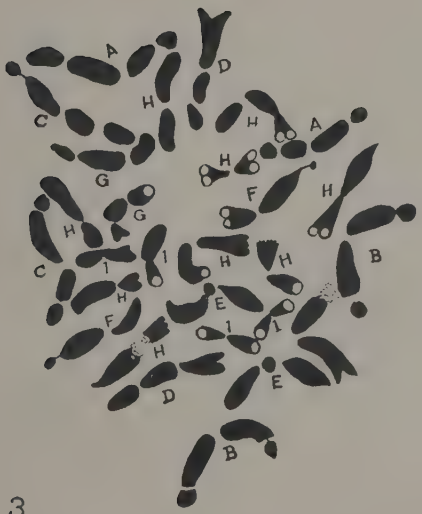

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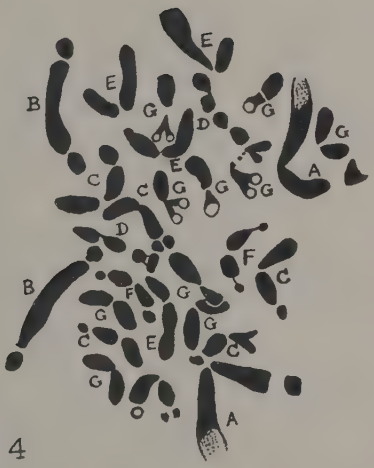

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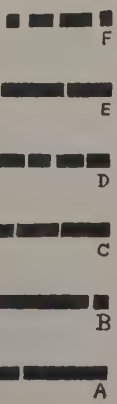
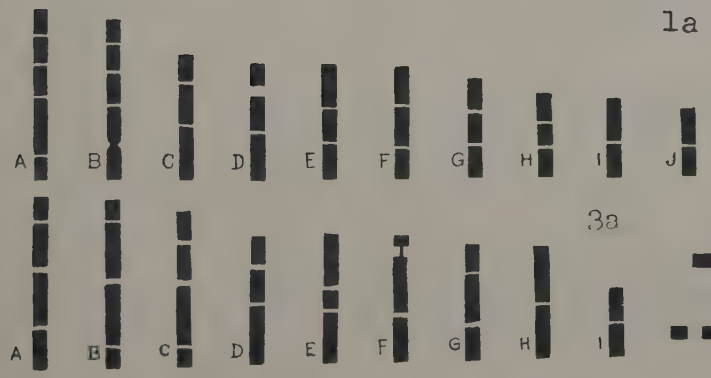
2a

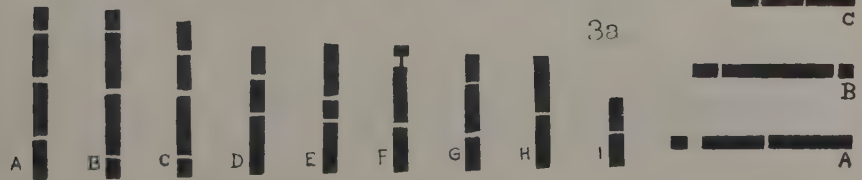
4a



1a



3a



The following types could be seen:

Type A — One pair of long chromosomes with nearly median primary constriction. The long and short arms are provided with nearly median secondary constrictions;

Type B — One pair of long chromosomes with median primary constriction and provided with two secondary constrictions at the distal ends of the two arms respectively;

Type C — One pair of long, but comparatively shorter chromosomes with nearly median primary constriction with one subterminal secondary constriction at the distal end of the comparatively long arm and with another secondary constriction placed nearly median to the short arm;

Type D — One pair of medium-sized chromosomes with nearly median primary constriction and median secondary constriction at the comparatively long arm;

Type E — One pair of medium-sized chromosomes with a nearly median primary constriction and provided with a secondary constriction placed very near the primary one towards the longer arm;

Type F — One pair of medium-sized chromosomes with a nearly median primary consticti on and with a small SAT placed at the distal end of the comparatively long arm;

Type G — One pair of medium-sized chromosomes with a nearly submedian primary constriction and with an almost median secondary constriction at the comparatively long arm;

Type H — Five pairs of medium-sized chromosomes with more or less median to submedian primary constrictions;

Type I — Two pairs of short chromosomes with median primary constrictions.

4. *Caladium bicolor* Willd. var. *verschaffeltii*

Somatic chromosome number of the species is 28 (Fig. 4). Besides this normal number, 30, 31, 32 chromosomes are also found in different plates.

The chromosomes of the normal metaphase plate may be classified as follows (Fig. 4a):

I. One pair of longest chromosomes.
II. One pair of long chromosomes.
III. Six pairs of medium-sized chromosomes.
IV. Six pairs of short chromosomes.

The size difference of the chromosomes ranges from 2.77 μ to 7.22 μ.

The following types may be seen:

Type A — One pair of very long chromosomes, longest in the complement with a median primary constriction and a big satellite at the end of one arm;

Type B — A pair of long chromosomes with a sub-terminal primary constriction and a sub-terminal secondary constriction at the end of the long arm;

Type C — Three pairs of medium-sized chromosomes with nearly median primary constriction and a satellite at the end of the comparatively long arm. The SAT-segment varies slightly in size in different pairs;

Types D, F — Two pairs of medium-sized chromosomes with median primary constriction and two secondary constrictions at the two arms. It is to be noted that the primary constrictions in these chromosomes are remarkably prominent and secondary constrictions too in F-pair.

Type E — Two pairs of medium-sized chromosomes with a nearly median primary constriction;

Type G — Five pairs of short chromosomes with median to submedian primary constriction.

In addition to normal 28 chromosomes distinguishable into 14 pairs as stated above, the species show a remarkable peculiarity in having altered karyotypes in the same individual with normal chromosome number. In addition to this again, cells have also been encountered having chromosome number different from the original diploid complement with the karyotypes altered. The following are a few of such peculiar cases met with:

Fig. 5 — It is a 28-chromosome plate with one member of A and B missing and replaced by two extra chromosomes A_1 and B_2 - A_1 having supernumerary constrictions and B_2 not being nucleolar.

It is quite likely that fragmentation across the secondary constriction segment on a member of B-type and the translocation of the secondary constriction segment on a member of A-type of chromosome has resulted in types A_1 and B_2 (shown in Fig. 5a).

Fig. 6 — It is also a 28-chromosome plate, but having a member of A-pair missing and replaced by X_1 - chromosome having secondary and primary constrictions placed equidistant from the centre and another extra chromosome with nearly median primary constriction (A_1) and having the end of one arm attenuated.

It seems possible that the missing member of the A-pair has undergone fragmentation at the secondary constriction region resulting in A_1 - type. The fragmented part seems to have been translocated on a short chromosome (G) resulting into type X_1 (shown in the Fig. 6a). The total amount of chromatin in length of A and S chromosomes corresponds approximately with lengths of A_1 and X_1.

Fig. 7 — Shows a metaphase plate with 30-chromosomes in which one member of A-pair is missing and two chromosomes belonging to C-type could be found extra. In addition, another chromosome with nearly submedian primary constriction marked S is also new in the complement.

It appears (Fig. 7a) that S-chromosome is the result of fragmentation of one of the A-type and the survival of the centromeric fragment, the non-centromeric part with the secondary constriction undergoing degeneration. The extra chromosome of the C-type might be the result of non-disjunction and the resultant duplication of the latter.

PLATE II — Figs. 5-8 - *Caladium bicolor* var. *verschaffeltii*. Somatic metaphase plates with 28, 28, 30 and 32 chromosomes respectively with altered karyotypes in the same root tip cells. Figs. 5a-8a - Show the assumed changes undergone by the chromosomes in the respective plates (for details — vide text).

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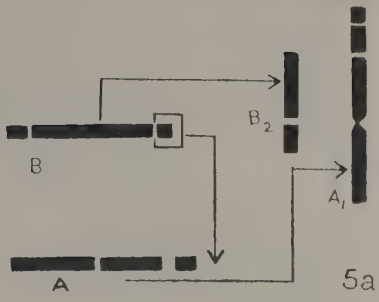

5a

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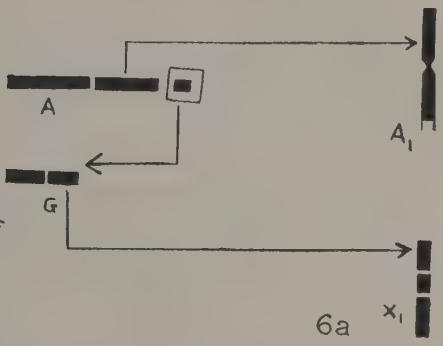

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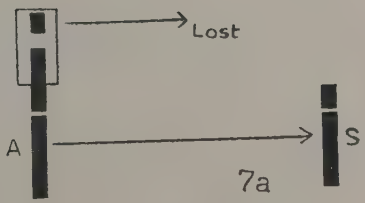
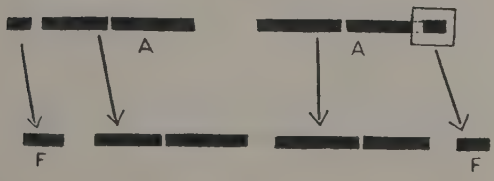
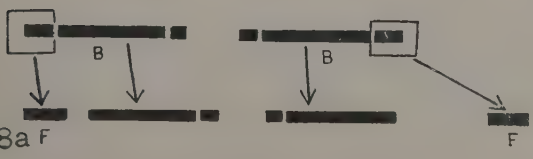


7a





8a

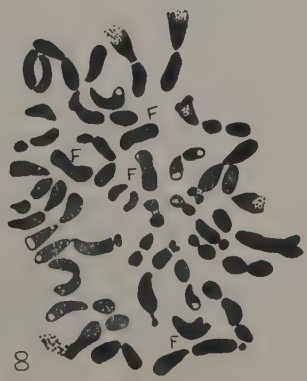


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Fig. 8 — It shows 32-chromosome plate where both A and B pairs of chromosomes are missing. The plate is also characterised by the presence of extra four medium-sized chromosomes, four fragments and two additional satellite chromosomes belonging to C-type.

It seems quite likely (Fig. 8 a) that the two pairs A and B have undergone fragmentation across the secondary constriction region resulting into four fragments (F) and four medium-sized chromosomes.

The occurrence of two additional chromosomes can be explained by assuming non-homologous interchanges involving two nucleolar chromosomes of C-type and two non-nucleolar chromosomes.

Fig. 9 — It shows a metaphase plate with 31-chromosomes. The plate is characterised by the absence of both the members of type A, substituted by one comparativelv smaller non-nucleolar pair (A_1, A_1) with nearly median primary constriction. Moreover, two extra chromosomes of the G-type and a new I-chromosome with submedian primary constriction and having a secondary constriction at one of the arms are present. The secondary constriction region of this I-chromosome is attached with the main body with a fuzzy gap in between and possesses also a constriction in its own body.

It seems plausible (Fig. 9a) that A-pair has undergone fragmentation away from the primary constriction and towards the secondary one, the fragmented part involving the region of secondary constrictions of both. One of these fragments seems to have been lost during divisional cycle the other being translocated to a member of G-type resulting in I-type of chromosome. Two extra chromosomes might be the result of non-disjunction of three chromosomes belonging to G-type.

Fig. 10 — It shows the metaphase plate with 32 chromosomes. The plate is distinguished from the normal one in the absence of one member each of A and B pairs

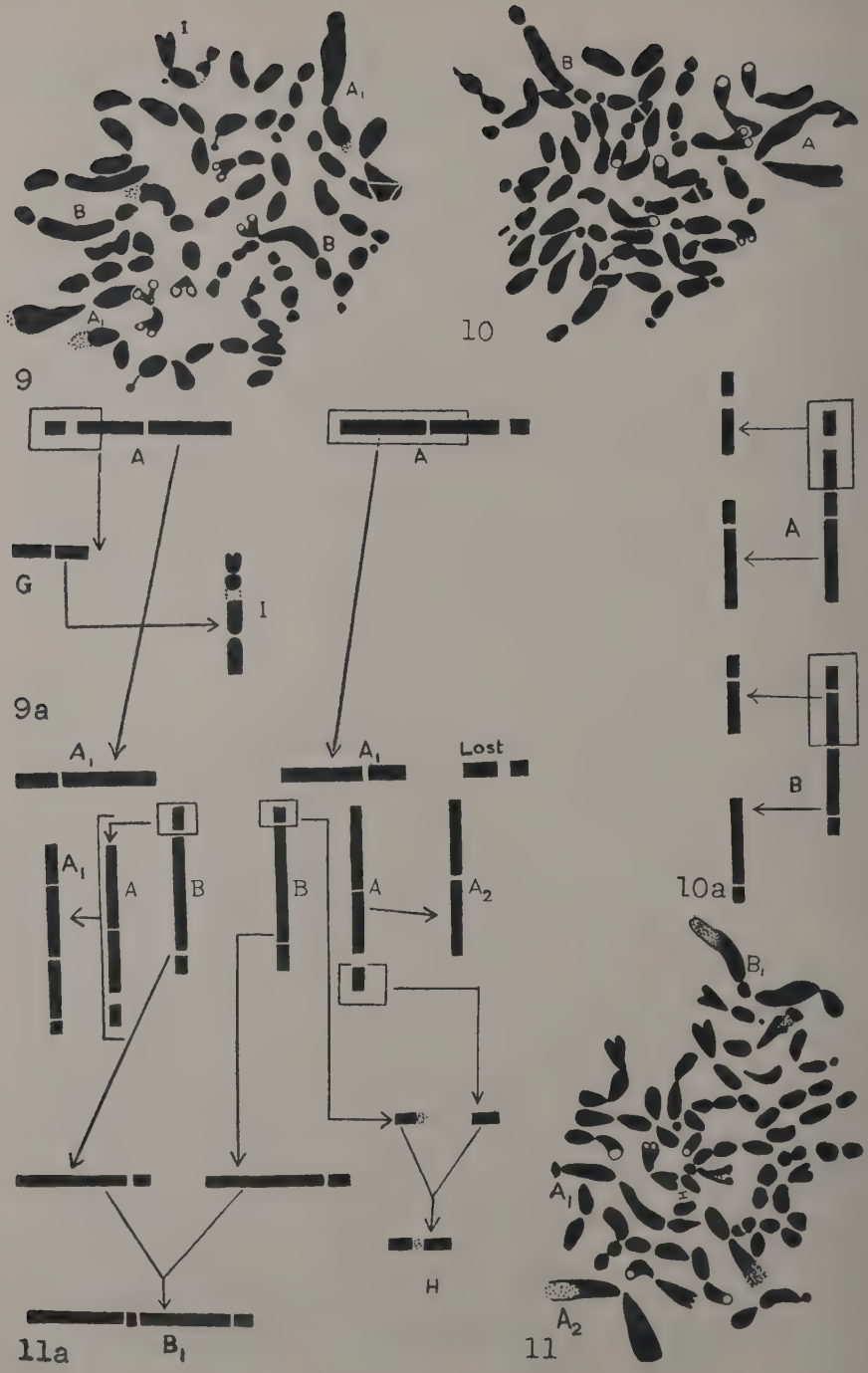
of chromosomes. In addition, there are present four extra medium-sized chromosomes with sub-median primary constriction and two chromosomes belonging to G-type.

It seems likely (Fig. 10a) that both A and B have undergone fragmentation near the middle of the arm resulting into four medium-sized chromosomes with submedian primary constrictions. The origin of two extra chromosomes can be assumed through non-disjunction in the previous cycle of division. If such fragmentation is assumed, then of the four medium-sized chromosomes resulting after such a process, two would be centric and the other two would be acentric. It is not possible, from their appearance in the complement, to ascertain their centric or acentric nature, since both primary and secondary constrictions look alike. Recent techniques following the use of oxyquinoline (TJIO and LEVAN, 1950; SHARMA and GHOSH, 1950; SHARMA and BHATTACHARJEE, 1952), coumarin (SHARMA and BAL, 1953) etc. are expected to reveal in these cases, the nature of the constrictions, whether centric or acentric.

Fig. 11 — It is a metaphase plate with 32 chromosomes. The plate is characterised by the absence of both A and B pairs of chromosomes and in the presence of the following extra chromosomes:

I. B_1-type — It is a long chromosome with median primary constriction with a secondary constriction very near the primary one and another secondary constriction at the end of the other arm. It is noteworthy that the total length in chromatin of the pairs A_1 A_1 and the translocated part of I (12.48 μ) falls a bit less than the amount of chromatin matter present in the original pair of chromosomes A (14.44 μ). This is easily accountable in view of the assumed loss of a fragment of this original pair.

II. A long chromosome (A_1) with median primary constriction and two secondary constrictions at the two ends;


I
A1
B
B
A1
9
B
A
10
A
A
G
I
9a
A1
A1
Lost
A1
A
B
B
A
A2
A
B
10a
B1
A1
A2
H
11a
B1
11

III. A long chromosome (A_2) with nearly median primary constriction;
IV. A very short chromosome (H) with unusually pronounced median constriction;
V. Four extra medium-sized chromosomes with secondary constrictions.

It seems apparent (Fig. 11a) that the A_1-chromosome is the result of translocation on one end of A chromosome, of a secondary constriction region broken from a B-chromosome. The residual part of B-chromosome containing the primary constriction has been attached with the other member of the B-chromosome at the end, the other end of which in turn has been fragmented resulting in B_1-chromosome. The fragmented part of B-chromosome, on the other hand, possessing the centromere seems to have undergone attachment with secondary constriction segment of an A-type chromosome which has been fragmented previously at that particular locus. These result into chromosomes H and A_2 respectively. A_2 chromosome thus becomes the residual part of the broken «A» chromosome. The fuzzy end possibly suggests the point of breakage. The four chromosomes with the secondary constrictions which are found extra in the complement can easily be accounted for if one assumes non-disjunction of several of the C-types.

Fig. 12 — It is a 30-chromosome plate resulting out of non-disjunction of two chromosomes.

In order to account for the origin of such altered chromosome plates, it is evident from the above paragraphs that reunion has been visualized to have taken place between broken and unbroken ends. We are fully aware that this is contrary to the accepted theory of breakage and reunion. But gradual experience in this laboratory on different varieties of plants, viz., *Scilla* (BHADURI and SHARMA, 1949), Amaryllids (SHARMA and GHOSH, 1952), where such phenomenon are often encountered and which can be accounted for

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PLATE III — Figs. 9-11 - *Caladium bicolor* var. *verschaffeltii*. Somatic metaphase plates with 31, 32 and 32 chromosomes respectively with altered karyotypes in the same root tip cells. Figs. 9a-11a - Illustrates the assumed mode of karyotype alteration (for details — vide text).

by none but such a process, leads us to believe in the occurrence of such a process in speciation. Reunion between broken and unbroken ends is possibly facilitated by the presence of telomeric heterochromatin whose sticky consistency seems to be an essential factor in this operation. The heterochromatic or broken nature of some of the end segments as observed has also been indicated in the figures (segments marked with striplings). The other alternative way of explaining this reunion is to assume the presence of minute breakages at the apparently unbroken ends. Though this assumption fits in well with the classical concepts, in absence of definite evidences in these materials in favour of this phenomenon, such a contention seems to be highly speculative in the present case.

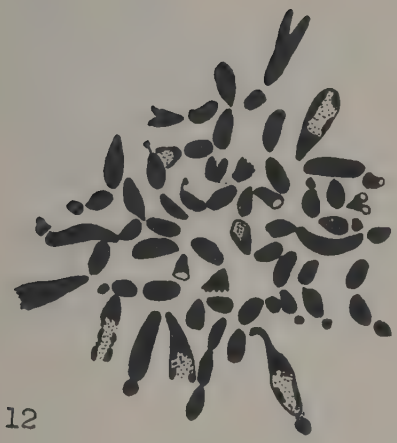
In addition to normal diplotene and diakinesis in a low percentage of cells showing 14 bivalents, P.M.C.s were observed containing one or two extra chromosomes within the same *loculus* (Figs. 23, 24). Clear 14 bivalents could be counted in metaphase (Fig. 27) and in most of the cases, especially in sideview, the longest pair of chromosome having characteristic configuration could be observed (Fig. 27 - «X»). Anaphase shows irregularities, showing inversion bridge (Fig. 25), the late separation of some of the chromosomes (Fig. 26) etc. Normal metaphase has been observed during second division showing 14 and 14 chromosomes in the two nuclei in only rare cases (Fig. 28). Pollen sterility has been found to be 90 %.

5. *Caladium bicolor* Willd. var. *Chantini*

The diploid chromosome number of the species is 28. In addition to normal plates, metaphases were observed with 27 as well as 30 chromosomes in 2-3 % of cases. The normal plates on classification reveal two pairs of long chromosomes, five pairs of medium-sized chromosomes and seven pairs of short chromosomes. The chromosomes could be classified into the following types (Fig. 13 and 13a):

Type A — One pair of long chromosomes having median primary constriction;

Type B — This pair of long chromosomes is characterized by the presence of nearly submedian primary constriction and a secondary constriction at the distal end of the long arm;



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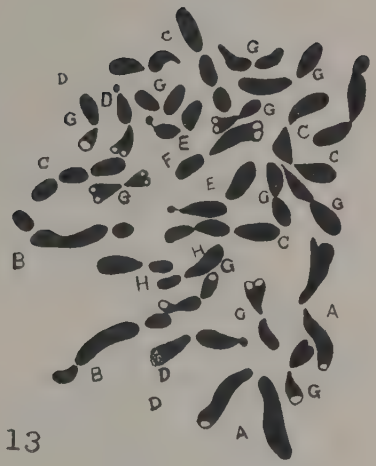

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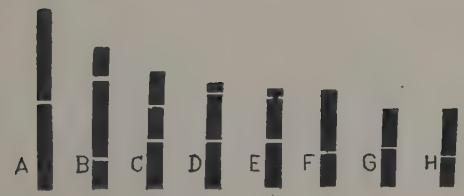

13a

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PLATE IV — Fig. 12 - Somatic metaphase plate of *Caladium bicolor* var. *verschaffeltii* showing 32 chromosomes. Figs. 13-15 - *Caladium bicolor* var. *Chantini*. Somatic metaphase plates showing 28, 27 and 30 chromosomes respectively. Fig. 13a - Idiogram drawn from Fig. 13.

Type C — Two pairs of medium-sized chromosomes with nearly median primary constriction and one secondary constriction placed medianly at the comparatively long arm;

Types D, E — Two pairs of medium-sized chromosomes with median primary constrictions and a very small satellite at the end of one arm; pair EE differs from DD in that the satellite here is provided with a minute thread joined with the arm of the chromosome;

Type F — One pair of medium-sized chromosomes with nearly median primary constriction;

Type G — Six pairs of short chromosomes with median primary constriction;

Type H — One pair of short chromosomes having nearly submedian primary constriction.

Fig. 14 — This is a plate with 27 chromosomes, one each of B and C types being missing and an extra chromosome B_1 with two secondary constrictions being present. Though the absence of members of B and C type of chromosome and the presence of B_1 type is quite prominent, the other chromosomes due to a bit of clumping (drawn spaced apart) do not yield their morphology with perfect clarity. Therefore in the present case, it is not possible to visualize the way of origin of the B_1 chromosome, though it needs no mention that considerable structural changes of chromosomes involving breaks and translocations are responsible for the origin of this new chromosome marked B_1 in the complement.

Fig. 15 — It is a metaphase plate with 30 chromosomes characterized by the presence of two extra chromosomes. The origin of the two extra chromosomes can safely be assumed through non-disjunction.

6. *Caladium bicolor* Willd. var. *unidentified* (Figs. 16-22)

Another spotted variety of *Caladium bicolor* with petiole absoletely black and lamina spotted black has also been cytologically

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PLATE V — Figs. 16-22 - *Caladium bicolor* var. unidentified. Somatic metaphase plate showing 30, 29, 30, 32, 33, 29 and 28 chromosomes respectively.

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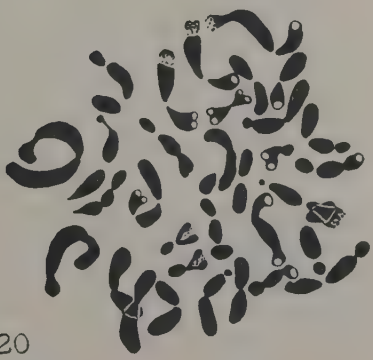

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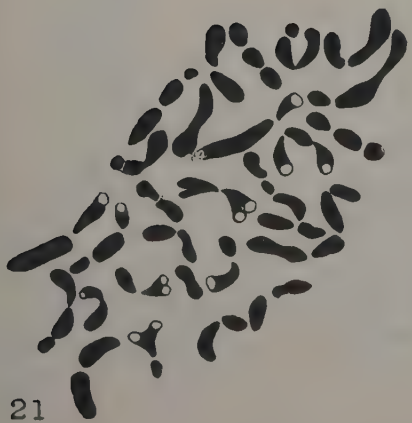

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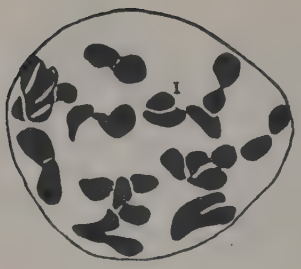
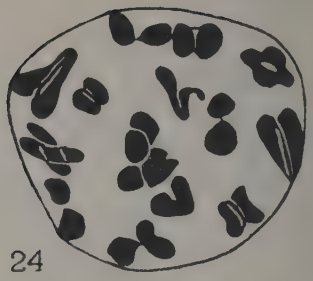
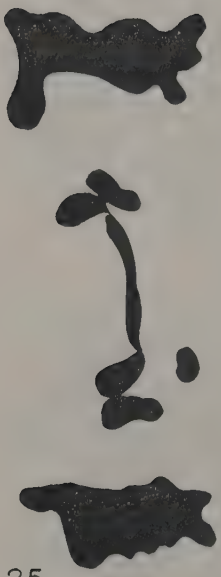


23



24



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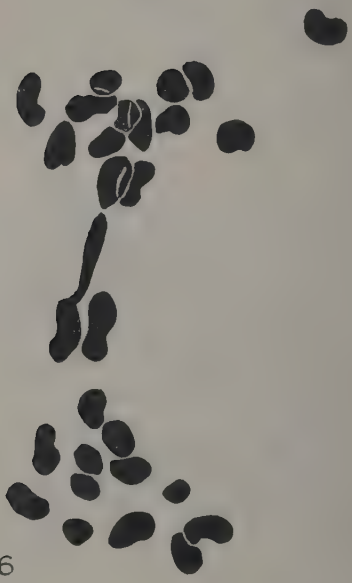

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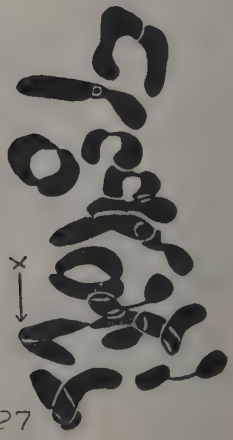

27

examined. This variety differs from *C. bicolor* var. *Varschaffeltii* but could not be identified here. As large number of plates with regular chromosome number could not be obtained, a thorough karyotype study has not yet been made possible. However, the presence of chromosome types A, B, C etc. shows much of similarity with *C. bicolor* var. *verschaffeltii* and points towards its affinity with the latter, though some of the types differ from that of *C. bicolor* var. *verschaffeltii*. Here too, a large number of plates with irregular chromosome numbers have been obtained, occurring within the same root tip.

In all such cases, with irregular chromosome number, no specific zones in the root tip could be found showing such irregularity. Rather these cells occur at random being distributed throughout the root tip tissue.

DISCUSSION

I. *General chromosome number and the nature of species.*

Though a critical investigation has not yet been attempted on the cytology of Aroids, the family has often been explored by various cytologists who have counted the chromosome number of its wide variety of forms (BOWDEN, 1940; DARLINGTON and JANAKI AMMAL, 1945 etc.). Different basic numbers have been reported in the family including X 8, 9, 11, 12, 13, 14 and 17. How far all these so-called basic sets represent divergent lines of evolution from the onset is yet to be investigated.

The present report, as noted, deals with four different genera, viz. *Alocasia, Colocasia, Caladium* and *Gonatanthus*. In all these cases, 28 have been found to be the somatic number of the species. Of them *Colocasia* and *Alocasia* have been investigated in the past, the reported somatic number of *Colocasia antiquorum* and *Alocasia macrorrhiza* being 28 and 26 respectively (DARLINGTON and

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PLATE VI — Figs. 23-28 - Meiotic figures of *Caladium bicolor* var. *verschaffeltii*. Figs. 23-24 - Diakinesis stage showing 14 bivalents and 1 univalent, and 15 bivalents respectively. Fig. 25 - Dicentric bridge with a fragment in first meiotic anaphase. Fig. 26 — Late separation of chromosomes in first meiotic anaphase. Fig. 27 - First meiotic metaphase (side view) showing 14 bivalents. Longest bivalent is marked « X » in the figure. Fig. 28 - Second meiotic metaphase showing 14 chromosomes in each of the two nuclei respectively.

JANAKI-AMMAL, 1945). The chromosome number in the former species has been corroborated in the present investigation too. The occurrence of 28 chromosomes in the body cells of *Alocasia fornicata* and 26 in an allied species *A. macrorrhiza* suggests probably the derivation of the latter from the former types through non-disjunction and union of reduced gametes. This finds support in the constitution of many other species where 14 chromosomes were observed to constitute the haploid set.

The report of 28 chromosomes of *Caladium bicolor* made for the first time during this investigation is also significant. The meiotic data where neither multivalent formation, nor secondary association has been noticed, show in all probability the diploid nature of the species concerned. The occurrence of irregularities in the P. M. C.s show possibly that some such irregular cells of the root tip survive, enter into the growing tip and take part in the formation of gametes.

It must be remembered, however, that the genus *Caladium* though differing in phenotypic character from that of *Alocasia* (particularly manifested in the presence of spotting on the leaf surface), resembles *Alocasia* in gross cytological structure as also the species of *Gonatanthus*. On the other hand, *Colocasia* though regarded by taxonomists as closely allied to *Alocasia* show wide difference in chromosome-morphology from the former. From a study of the cytological details, one is tempted to visualise the presence of at least two divergent lines of evolution operating within these genera, one including the genus *Colocasia* and the other, *Alocasia*, *Caladium* and *Gonatanthus*. Within these lines of evolution during later stages, structural changes of chromosomes can safely be assumed to have played a major role in the origin of different forms.

II. *Chromosomes with supernumerary constriction and their origin.*

It is evident that of the three genera studied, two are characterized by possessing a high number of secondary constrictions including supernumerary ones in the complement.

It is significant to note that though a critical investigation on the chromosome-nucleolus relationship with the aid of the Feulgen — light green technique (BHADURI, 1938) has not yet been made

possible, a large number of nucleoli has often casually been observed in the root-tips of these species.

The original concept of DE MOL (1928) that the nucleolar number is an index of polyploidy has already been questioned by several authors (BHADURI, 1942a, b, 1943; BHADURI and BOSE, 1946; BHADURI and SHARMA, 1946; BHADURI and KAR, 1948; SHARMA, 1947; CHAKRAVORTI, 1948). It has been ascertained that the increase in the number of the nucleoli can also be brought about apart from polyploidy through non-homologous interchanges involving nucleolar and non-nucleolar chromosomes. It is further claimed by BHADURI and others (l. c.) that there is a direct correlation existing between the maximum number of secondary constrictions and the maximum number of nucleoli of the nuclei. That the relative size-difference between nucleoli in a species is a constant character and homo-morphicity of the nucleoli indicates homozygosity as against hetero-morphicity for heterozygosity, has also been demonstrated.

In the light of the above evidences put forward by different authors. the secondary constrictions observed in the present case show the possibility of their being all nucleolar. If that be the case, the origin of such high number of secondary constrictions including the supernumerary ones undoubtedly can be visualised to have originated through non-homologous translocations involving nucleolar and non-nucleolar chromosomes. Whether the plants investigated are homozygous or heterozygous for such translocations cannot be ascertained at present pending a thorough investigation into the meiotic behaviour of the species. It is worthy of note that the plant is propagated mainly by vegetative means and as such irregularity during meiosis, if any, can have little effect on the evolution of phenotypes.

With due regard to the above interpretation of the origin of high number of nucleoli, another possibility too might be suggested.

In widely different families, the occurrence of high number of supernumerary constrictions has recently been observed (BHADURI and BOSE, 1946; CHAKRAVORTI, 1948 etc.). The present data taken in conjunction with the above evidence, leads one also to visualize the presence of basic sets with chromosomes having supernumerary constrictions in the complement. The suggestion though not amply documented seems to us to be quite a reasonable line of approach towards the problem of the origin of chromosomes with

such structural peculiarities and possibly also of the high number of nucleoli.

III. *Karyotype alteration and its significance.*

In addition to normal 28 chromosomes in *Caladium bicolor* var. *verschaffeltii*, nuclei with varying number of chromosomes with altered morphology have been observed in the same root-tip cells. Their origin has been assumed through fragmentation and translocations occurring spontaneously in the somatic cells. Such behaviour has been previously recorded in case of *Scilla, Musa* and *Zephyranthes* etc. (SATO, 1936; RAGHAVAN and VONKATASUBBAN, 1939; BHADURI and SHARMA, 1949; CHAKRAVORTI, 1948; SHARMA and GHOSH, 1954). The cause bringing about such disbalance in chromosome division is yet obscure. Habitat factor might play some role in bringing about the process and as such, investigations on individuals collected from diverse types of habitat is highly desirable.

In recent years, such somatic irregularities have often been encountered in plants propagated mainly through vegetative means (SHARMA and BHATTACHARYYA, 1954; SHARMA and BAL, 1954). Their occurrence in such specialised group of plants tempts one to conclude that in these cases, the balance between the chromosomes and the cytoplasm is not maintained in the somatic cells between individual nucleus and cell-cytoplasm but rather between the absolute amount of chromosome matter and cytoplasm within the tissue as a whole.

The significance of such karyotype alteration is far reaching. If such cells with altered karyotypes are present in the point of origin of the daughter corms as well, new phenotypes can easily be produced. The occurrence of horticultural and wild forms of Aroids in nature needs mention in this connection. Significantly, however, it has been noted that the unidentified variety of *Caladium bicolor* with black petiole, which shows slight deviation from this one, reveals cytological similarity with *C. bicolor* Willd. var. *varschaffeltii* specially in the gross morphology of the chromosomes. The origin of the latter type could be assumed through *C. bicolor* Willd. var. *verschaffeltii* types or from a common type mainly through structural changes of chromosomes in cells entering into the growing point of the corm, the resulting corm being differentiated from the rest in its chromosome complement forming a new individual with altered chromosome morphology. This pro-

vides them with a new method of speciation in plants, specially in those groups where propagation is carried out mainly through vegetative means. A critical search into their nuclear nature involving also a comparison of all the allied types of horticultural and wild forms of Aroids is expected to reveal as to how far the above suggestion is coincident with the actual course of events.

Such irregular nuclei which are no doubt regular abnormalities in the life-cycle of an organism, however, enter into the formation of the germ cells, as noted by the high percentage of irregularities in meiosis. But their survival through sexual reproduction seems to be very remote as evidenced by practically 90 % sterility of the pollen grains.

It is significant that cases have been encountered where both centric and acentric chromosomes result out of fragmentation. The difficulty of locating primary and secondary constrictions specially in these cases have further been pointed out. In recent years, the use of oxyquinoline (TJIO and LEVAN, 1950; SHARMA and GHOSH, 1950; SHARMA and BHATTACHARJEE, 1952) has opened up new possibilities in this line of investigation. The chemical has been demonstrated to to possess the property of exaggerating the centromeric regions even bringing out with clarity the centromeric granules. The application of this chemical in such types is expected to reveal the nature of the constriction, whether primary or secondary.

The present observations also bring about controversial issues whether acentric fragments as found in several plates can acquire a centromere of its own or not. In recent years much discussion has been made on this aspect and interesting observations have been made such as in species of *Luzula, Musa* etc. whereby such possibilities in certain cases has been pointed out (CASTRO, CAMARA and MALHEIROS, 1949; CAMARA, 1951; BHADURI and BOSE, 1946; CHAKRAVORTI, 1948). Undoubtedly, the theory of CAMARA *et al.* might be taken advantage of in solving the problem, but it seems better to reserve opinion regarding this issue in so far as Aroids are concerned, pending confirmatory evidences from diverse groups of plants, evidencing universal applicability of this process in speciation.

SUMMARY

I. A detailed karyotype investigation has been carried out of six different species and varieties of Aroids, viz. *Alocasia fornicata*, *Colocasia antiquorum* var. *nymphaefolia*, *Caladium bicolor* var. *verschaffeltii*, *C. bicolor* var. — an unidentified variety of the same species, *C. bicolor* var. *Chantini* and *Gonatanthus sarmentosus*, the somatic chromosome number in all cases being found to be 28.

II. In addition to normal chromosome complement in *Caladium bicolor* var. *verschaffeltii*, cases were found with altered karyotypes within the same individual which has been assumed to have originated through fragmentations and translocations.

III. On the basis of cytological data, the presence of two different evolutionary lines among these genera, one including *Alocasia*, *Caladium* and *Gonatanthus* and the other *Colocasia* respectively, has been suggested.

IV. The origin of supernumerary constrictions encountered in some of the species investigated has been discussed.

V. The importance of karyotype alteration of the somatic tissue in the evolution of species has been pointed out.

ACKNOWLEDGEMENT

We wish to express our sincere thanks to Prof. P. C. SARBADHIKARI, Head of the Dept. of Botany, Calcutta University, for kindly permitting one of us (N. K. D.) to work in this laboratory.

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CHROMOSOMAL ABERRATIONS IN OATS, *AVENA SATIVA* L. [1]

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INTRODUCTION

THE importance of chromosome aberrations in cytogenetic studies has been demonstrated many times in recent years. Of the various cytological anomalies which are useful in locating genes in both theoretical genetics and in practical breeding, the whole-chromosome deficiency is perhaps the most generally and reliably usable kind. The value of such deficiency — obviously confined to polyploid plant species — was first described by CLAUSEN (1926, 1941), who pointed out the value of monosomic strains in determining the linkage relationships within a species. CLAUSEN noted that these techniques, such as the monosomic technique which he developed in *Nicotiana*, could not be used with diploid species because the necessary deficient gametes could not function with whole-chromosome deficiencies.

The «deficiency» methods have been used successfully to determine on which chromosome several specific genes were located. CLAUSEN located several genes in *Nicotiana Tabacum* by the monosomic method. In the cultivated cereals the nullisomic method has been used with similar success. Thus, the *sphaerococcum* gene was analyzed by SEARS (1946), the three genes concerned with awns by O'MARA (1947), a locus concerned with resistance to stem rust by SEARS and RODENHISER (1947), and several other genes by UNRAU (1950).

The preceding instances have been concerned chiefly with whole chromosome deficiencies as methods of locating loci in

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(1) Part of a thesis submitted in partial fulfillment of the requirements for the Ph. D. degree at the Iowa State College, Ames, Ia., U. S. A.; Journal Paper No. J-2552 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1181.

(2) Scholarship from the Instituto de Alta Cultura, Lisboa, Portugal.

specific chromosomes. The deficiencies have various other uses, however, both practical and theoretical. Among the practical uses the most important is that emphasized by MORRISSON (1953), who said that once a gene had been found in a known chromosome, that gene could be transferred to other varieties and strains without the necessity of testing for the presence of the gene, a most important advantage if the gene concerned a physiological or pathological trait not easily or always recognizable. A most important theoretical use is the determination of homeologous relationships, in contradistinction to the usual homologous relationships.

Whole chromosome deficiencies are known in several species. In *Nicotiana Tabacum* all twenty-four monosomics are known and have been described by CLAUSEN and CAMERON (1944). In *Triticum aestivum* (= *T. vulgare*) all twenty-one nullisomics have been described by SEARS (1952). In *Avena sativa* three monosomics were discovered by chance, one by HUSKINS (1927) and two by PHILP (1935, 1938).

The value of such cytological anomalies has been demonstrated. What is needed is a reliable and practical method for inducing whole-chromosome deficiencies in species which will tolerate such deficiency. The present study is concerned with the production of monosomics in cultivated oats, *Avena sativa.*

REVIEW OF LITERATURE

Although oats have been analyzed and studied in both agronomic and botanic studies, very little of the available information is related to chromosome deficiency. One part of the general problem of speciation and phylogeny in *Avena,* however, is very closely involved with deficiency. This is the relationship between *Avena sativa* or *A. byzantina* with *A. fatua.*

Winge's observations, reported by HUSKINS (1925), concluded that speltoid wheats could arise as a result of anomalous conjugations and disjunctions such that plants could occur which had an excess of some chromosomes and a deficiency of others. This theory of origin could also be applicable to fatuoid oats. Both *T. aestivum* and *A. sativa* are hexaploids in which homeologous associations could occasionally occur, and in which duplications and deficiencies of whole chromosomes could be compensatory (SEARS, 1944). The disimilarities between wheat and oats, however,

make necessary an independent investigation in oats before the parallel can be accepted.

Later, HUSKINS and FRYER, 1925, presented a large and complete review of the literature up to that date and concluded that natural crossing might occur occasionally between *A. fatua* and *A. sativa*, the progeny of such crosses being in practically all cases very different genotypically from the commonly occuring type referred to as false wild oats; and, therefore, they suggested the name «*Avena sativa* mut. *fatuoid*» for all homozygous forms of false wild oats, replacing the misleading description of Haussknecht of these forms as «*Avena fatua* var. *transiens*». In brief, they said, evidence from literature indicates that false wild oats originate from *A. sativa* varieties through some spontaneous change occuring in a gamete with chances for both heterozygous and homozygous forms to occur, directly from the normal.

HUSKINS (1927) observed in *A. fatua* a very regular meiosis with the chromosomes splitting practically simultaneous in the first anaphase, but occasionally laggard and vagabond chromosomes were found in *A. sativa*.

The segregation of the heterozygous fatuoids presented in his paper and for this particular material shows deviations that were attributed to selective elimination of fatuoid male gametes. Although the results were very variable, the percentage was usually greater in homozygous and heterozygous fatuoid segregates than in normal sibs.

Three types of fatuoids were here described. Those segregating 1 : 2 : 1, from 42 chromosomes plants, lagging higher than in normal plants and showing micronuclei; 41 chromosomes plants from variety *Victory* and others, segregating 1 : 1 plus homozygous dwarf fatuoids with 40 chromosomes, forming all sorts of irregular arrangements, up to octads; and those from variety *Kanota* with 44 chromosomes in the homozygous fatuoids, dwarf and sterile, the normal plants having 42 chromosomes.

The author stated that the fatuoids presenting genic segregation in normal fashion were formed due to the duplication of the chromosomes of the fatuoid type by allosyndesis and that those presenting diverse proportions in the segregation were due to the formation of micronuclei and sequent loss of the univalent, giving origin to plants with 41 and 40 chromosomes. In 1928 he found

out that the dwarf and sterile 40-chromosome fatuoids with irregular meiosis were deficient in both C chromosomes.

NISHIYAMA (1929) stated that the various studies on *Avena*, morphological and geographical (Trabut), serological (Zade), cytological (Kihara), hybridization studies (Tschermak), as well as investigations on immunity to infectious diseases (Vavilov), have given the same phylogenetic relationship reported by Thellung.

In a series of hybrids to study the affinity between chromosomes of the different species he presented the following results: In the cross between *A. barbata* Pott × *A. strigosa* Schreb., 73 % had 7 II, 25 % had 8 II, 2 % had 9 II, and 43 % had 1 + (3) III. In the cross of the same species by *A. fatua*, in one year 5.98 ± 0.147 II, in the other 6.78 ± 0.145 II, and by *A. sterilis* 9.45 + 0.148 II. In the following crosses the number of univalents from 1 to 4 was observed 5 % in *A. fatua* × *A. sativa*, 16 % in *A. fatua* × *A. sterilis*, 29 % in *A. sterilis* × *A. byzantina* and 33 % in *A. sativa* × *A. byzantina*.

In his other paper (1931) NISHIYAMA observes three modes of abnormal meiotic process in 40 chromosomes plants (a) of PMC with a number of bivalents, a few having no bivalents, all scattered in the whole spindle at the beginning of the heterotypic metaphase. The bivalents arrange themselves on the equator and univalents, not all, pass also to the equator and split longitudinally, the halves going to opposite poles. Before all lagging chromosomes reach the poles, daughter nuclei form their membranes so that micronuclei are often found at interkinesis; (b) when the PMC have a few or no bivalents. Most of the univalents are not divided into two halves though they clearly exhibit longitudinal splittings. The chromosomes are continuously spread between the two poles and gradually grouped in two masses of chromatin differing extraordinarily in quality and quantity. Sometimes, they form a large nucleus as a kind of restitution nucleus or present many chromatic bodies outside the main nuclei, (c) PMC without bivalents. These chromosomes, although with longitudinal splittings, do not divide and form restitution nuclei.

In plants with 41 chromosomes he observed three types of quartets: A with 2(20), 2(21), B with 2(20), 1(21), 1(20) + 1(1); C with 2(20), 2(20) + 2(1). Considering the two types (20 and 21) he obtained the ratio 6:1 which might be applied to the formation of egg cells, giving the frequency of zygotes as

36(40), 12(41), 1(42). The average sterility of the 41 chromosomes plants being 57.24 % and if it be assumed that the sterility would be caused entirely by the death of 40 chromosomes zygotes, the ratio in the resultant progeny will be converted as 8(40), 12(41), 1(42), in fair agreement with the observed and expected numbers.

From the literature, three main theories have been proposed for the interpretation of the mode of origin of fatuoid oats, (1) the chromosome aberration hypothesis, (2) the mutation hypothesis, and (3) the natural crossing hypothesis. It was concluded, according to the hypothesis first mentioned, that the occurence of chromosome irregularities is not necessarily dependent upon the fatuoid type of the grain characters, and that faulty conjugation of chromosomes may not be caused by one specific chromosome, but probably by several ones at least. The fatuoids may be classified in two series, those produced by the mutation of a gene complex in the C chromosome, and those arising through chromosome aberration.

PHILP (1933) presented his conclusions from a study of a hybrid between *A. sativa gigantica* × *A. fatua* and reported a family consisting of 9 green plants and 61 albino seedlings. From these green plants, 2 were weak and sterile, 6 gave an excess of albinos over greens and 1 gave a ratio of approximately 3 greens to 1 albino. The green plants of this latter family tested again had one-third breeding true green, two-thirds segregating 3 greens to 1 albino and a few had approximately 1 green to 6 albinos. Among the 1:6 type variegated plants were striking in number.

Bivalent formation was the rule in the F_1 hybrid, which indicated a considerable degree of homology between the chromosomes of the two species. Abnormal pairing, chains of 4 and 1 or 2 univalents were occasionally observed in F_1 and F_2 . No crossing over occurred within the *sativa* complex in the hybrids with *A. fatua* because differential segments in the C chromosomes of the hybrid inhibit it.

Following the pattern given already by NISHIYAMA, HUSKINS (1933) believed that not only fatuoids but also speltoids and other aberrant forms of oats and wheat arise as quantitative changes in the chromosome complement which range from whole chromosomes to segments on the limit of visibility. He classified the fatuoids as: A — with 42 chromosomes and segregating 1:2:1 with classes of

nearly equal vigor, B — with 41 chromosomes and segregating as a monosome, C — with 41 or 43 chromosomes, a class including all the other forms.

He suggested that chromosome loss or rearrangement might uncover «*fatua*» genes which normally are hypostatic, and that this uncovering might be and usually is accompanied by changes which are not relevant to the fatuoid condition but are linked with it, i. e., duplication, deficiency, etc.

In the same year, HUSKINS and HEARNE (1933) concluded that since some 40-chromosome plants regularly form 20 II, the chromosome missing in dwarf homozygous fatuoids and speltoids must carry a factor or some factors controlling chromosome pairing. As we have seen before this factor is supposed to be carried by chromosome C.

In fact, PHILP (1935) found 40 chromosome green plants with normal meiosis, quite vigorous and fertile. The chromosome missed here was called V_1 chromosome and its behavior seemed to be different from that of the C chromosome in the monosomic plants. It was included in the nucleus of the gametes somewhat less frequently and with more variation in frequency than the C chromosome. The mean frequency of 21 chromosome pollen grains for three 41 chromosome plants was 14.1 % and 10.1 %, respectively, for the C and V_1 chromosomes.

In the material from which he drew these conclusions he found also that polymeric factors control chlorophyll production and that these factor pairs lie in homologous chromosomes of different sets which do not pair normally. The abnormal pairing of these chromosomes in the hybrids is the reason for the segregation of pure recessive albino types with abnormal inheritance, due to the loss of chromosomes or crossing-over between the dissimilar homologues following their exceptional pairing.

NISHIYAMA (1936) also assumed partial homology between A and B genomes of the hybrid AAAA × AABB; i. e., enough homology to permit nearly full pairing under obligatory circumstances. He secured as many as 13 II.

LATER, PHILP (1938) reported again another monosomic type from the cross *A. fatua* × *A. sativa gigantica* which also produced nullisomes. He studied leaf width and found a range of variation almost continuous. Nevertheless he concluded that plants whose seedling leaf width was 3 mm or more had 41 chromosomes and

that ones with less than 3 mm had 40 chromosomes (less vigorous and less fertile). The heterogygous broad-leaved plants did not segregate in a simple Mendelian manner, since they gave an excess of recessives, because the genes for broad leaves were carried in the involved L chromosome and this chromosome was only included in the pollen nucleus in about 4.25 %-7.75 % of cases. Assuming the same percentage in the female side, the expected ratio of broad and narrow-leaved plants was calculated and the expected results were close enough to the observed results to justify the conclusion that this was the explanation of the aberrant segregation.

It seems then that there is a pair of genes in each one of the genomes affecting the width of the leaves. The L chromosome is different from the V and C chromosomes. There is also crossing over between chromosomes of different genomes, which is proved by the formation of one fragment of chromosome V and the presence of an inversion.

The variation in the segregations is perhaps due to the environment, to the loss of zygotes of 40 chromosomes, or to the phenomenon of «certation».

In other species the same aberrant types have been obtained experimentally. The only extensive work was that of KIHARA and NISHIYAMA in *Triticum* and the analysis of the monosomics fluted and corrugated in *Nicotiana Tabacum* by CLAUSEN (1926) and CLAUSEN and GOODSPEED (1926). In these cases n-1 pollen grains rarely functioned and selfing gave substantially the same results as backcrossing, with rapid return to parental chromosome number.

KIAHRA, and later NISHIYAMA (1928) reported that in *Triticum vulgare* and *T. spelta,* male and female gametes both function and nullisomics having only 20 II may be obtained. By crossing monosomics involving different chromosomes, plants having 19 II + + 1 A + 1 B were also obtained.

LAMBERTS (1932) obtained seven monosomics in *Nicotiana rustica* with varying percentage of functional n-1 gametes according to the individual cases. Owing to lagging, the per cent was usually over 50. However, only 31 % of the functional gametes were n-1. This was not the result of inviability because about 95 % of the seed germinated and practically all the plants survived. Only three types were transmitted in appreciable frequency through the pollen. Nullisomic plants (2n-2) were not obtained even after selfing with a limited amount of pollen on shortened styles.

SEARS (1939) reported five monosomics obtained from haploid wheat pollinated by diploid. These haploids were obtained from a culture of *Chinese Spring* wheat pollinated by *Secale cereale*, and their identification made through the corresponding nullisomics, using morphology of the univalents and pairing of the chromosomes with those of *Triticum durum*. Later (1941) he gave a description of these five nullisomics.

CLAUSEN (1941) isolated twenty or more monosomic types of *Nicotiana Tabacum* differing from one another in morphological features but also differing in other features, as per cent of ovule abortion, character of pollen samples, per cent of transmission, characters of the unpaired chromosome in meiosis, etc. These were obtained mainly by crosses of asynaptic female × normal male.

SEARS (1944) reported seventeen nullisomics obtained from the respective monosomics in percentages varying from 1 to 10 %. Among them ten or eleven were fertile to some extent as both male and female, five or six were fertile on the female side only, and one was fertile only on the male side. Under the most favorable conditions of environment, few of the monosomics differed appreciably from normal but under less favorable circumstances there might be observed some departure from the normal type in the direction of the nullisomic.

CLAUSEN and CAMERON (1944) reported the twenty-four monosomic types of *N. Tabacum* which differed from normal and from one another in a specific ensemble of quantitative morphological features, and might be classified accurately on the basis of their morphology, given a sufficiently uniform genetic background.

HUSKINS (1946) presented a review of the fatuoid, speltoid, and related mutations of oats and wheat and reaffirmed the conclusion, already emphasized, i. e., that they arise through chromosome aberration, and their diverse genetic ratio-types are determined by their particular chromosome constitutions.

MATERIALS AND METHODS

The selection of a suitable strain of oats was important for the purposes of the present work. Various requirements had to be met, among them, earliness, erect short straw, and large anthers were the most important. Earliness was necessary because more than one crop a year had to be secured. Earliness is especially

important because under the greenhouse conditions during hot weather the fertility is highly affected. Stiff straw is necessary because straw which lodges and falls through the tillers of neighboring plants may be erroneously recorded. Large anthers are a characteristic useful only to the cytologists.

All those points were considered in the selection of the strain used. From different varieties then tested MISSOURI 04047, *Avena sativa* L., was as accepted as the best.

The cytological examination of plants by the usual method, i. e. the examination of meiosis at the first division, proved to be too tedious for satisfactory progress. Too much effort had to be employed in securing samples at precisely the proper time. In addition to this difficulty was the added one of finding metaphases in the selected samples. Since oats have a panicle, the selection of florets at the proper stage is much more difficult than in a plant like wheat, which has its florets in a convenient sequence on a spike. The procedure was simplified by making no attempt in the first sample to include anthers with metaphases. The only requirement for the first sample was that it include tetrads. The tetrad stage lasts a comparatively long time, and this long period permits more infrequent sampling and a larger number of samples. After the first samples had been examined at the tetrad stage, a second sample was taken from those plants which had micronuclei in the tetrad components. This second sample was carefully chosen to make the inclusion of anthers with metaphases as certain as possible. The procedure of double sampling made possible the discarding of normal, and therefore irrelevant, plants as simple and inexpensive as is now conceivable. The first sample allowed for the reduction of the population to the useful and interesting residium with a minimum of time at the microscope. In many instances the sample with tetrads also had usable metaphase stages. Obviouslynot all tetrads with micronuclei come from metaphases with the monosomic condition. The monosomic condition must be distinguished from inversions, asynapsis, and other phenomena which can produce micronuclei at meiosis, (Fig. 13).

The microspores were examined by the acetocarmin technique for smears, under a Bausch and Lomb microscope, objective 97, ocular 10. Pictures were taken with a Bausch and Lomb photomicrographic camera with Kodak panchromatic plates 3 1/4 × 4 1/4

in., magnification $\times 1000$. Drawings were made with a Spencer camera lucida and printed with a magnification of $\times 800$.

The monosomics of oats seem to be more difficult to distitguish morphologically than those of wheat because under the most favorable conditions of environment few of the monosomics differ appreciably from the normal plants. Their identity will have to be established by test crosses with other monosomes, or if possible, with nullisomes. All plants were grown in 6″ pots except those in Figs. 8 to 11 which were in 4″ pots.

The number of monosomics obtained was satisfactorily high. It is possible that some variation in the dose of 300 r units used will produce more monosomes but it could also produce, if increased, more of the other abnormalities which can only be separated at a considerable expense in time and effort. The radiation took place when the most mature florets were about to shed pollen but before actual anthesis had occurred.

EXPERIMENTAL RESULTS

The seed secured from irradiated eggs was planted in the greenhouse because such material is too valuable to be risked in the field. The first generation of the treated gametes — that which came directly from the treated eggs — consisted of 279 plants. Of these 37 or 13.3 % were retained as suspected monosomics because they had in their tetrad components one or two micronuclei. Not all of these plants were fertile. Those that did set seed and could be examined again in the following generation included plants which had micronuclei produced by cytological anomalies other than univalents which were monosomic. Such anomalies were simple partial asynapsis, inversions, and other irregularities. The final yield of monosomic plants was 20 or 7.2 %, which is a yield that is well within the range of practical use. The yield might have been even better than this if the plants had been grown during a more favorable season. Since they were grown under far from optimum conditions in the first generation from treatment, some of the selected plants set very few seed. That the only viable seed should be normal under these conditions is not surprising. Under better conditions some of these plants might have produced monosomic as well ás normal, if not nullisomic, seed.

Under the conditions of the second generation, when the

progenies of monosomic plants were grown so that morphological comparisons could be made, the same limitations existed, *id. est* the season was not favorable. The original ten monosomics were studied under better conditions, when the distinctions between disomics and monosomics, and nullisomics, can easily be made. The second sample could not be observed at this season, and so the distinctions will have to be made during future generations of the material. The original ten came from exploratory treatments and somewhat confounded samples, and thus were not reliable as indicators of the frequencies with which the monosomics could be expected. Although more is known about them individually, they are not part of an attempt to measure frequency. The original sample was useful in determining the segregation or transmission characteristic of the monosomics. The first sample was available for study during a normal season. Five progenies which came from monosomic plants were grown in the greenhouse under favorable conditions and classified. The frequencies of nullisomic plants were 6.5 % for nullisomic *a*, 3.0 % for *c*, 32 % for *f*, 34.8 % for *m* in one season and 46.0 % in another, making an average of 43.7 %, and 2.1 % for nullisomic *o*. An attempt was made to secure similar information for the twenty monosomes secured in the present investigation but unfavorable conditions made the data secured unreliable and so they have not been presented here. Differences in growth for both seasons, i. e. normal and late, may be seen by comparing Figures 6 and 9. These two illustrations are of nullisome *m* in both seasons. Other monosomes and nullisomes may be seen in figures 2 to 11.

The dose of 300 r units was both effective and efficient in the present experiments. The conclusion does not seem justified, however, that this is the optimum dose for producing this particular cytological anomaly desired, i. e. monosomics for the production of nullisomics. Obviously this could not be the ideal dosage except for a most improbable choice of the perfect treatment. The dose does seem to be one which can be used with predictable and dependable results. Further trials at higher dosages seem to be desirable.

DISCUSSION

The purpose of the present study was to secure some measure of the efficiency of the method used to extract monosomic plants, to record some of the characteristics of the monosomes and nullisomes now available, and to preserve the new monosomes induced in the experiments. The limiting factor in such experimentation has always been a lack of deficiencies. The methods which have been used are not generally applicable, because they are associated with unusual properties of the species in which they have been used. The method used by CLAUSEN in *Nicotiana Tabacum* requires that the species in which the monosomics are desired form fertile hybrids in which univalent chromosomes occur at meiosis, or that an asynaptic strain be available. Such a strain was available in a mutant, *pale-sterile*. The hybrid used was between *N. Tabacum* and *N. sylvestris*. Such conditions are not to be had in most species in which the monosomics could be useful.

The other method which has had success is that of pollinating a haploid plant with pollen from a normal plant. This is the method used by SEARS in *Triticum aestivum (= T. vulgare)*. The haploid is partially fertile owing to the formation of some type of restitution nucleus. Such eggs as do function, however, are not wholly normal because the restitution process is not perfect. Deficient eggs are formed and these produce, when united with normal sperm, zygotes which are monosomic for one or more chromosomes. This method cannot be used with all species because haploids are generally rare and cannot be produced at will. With few exceptions haploidy may be considered a wholly fortuitous occurrence.

In those instances in which hybridization is not possible some other method of induction must be used. Since X-rays fragment the chromosomes in various manners, their use for the present purpose seemed indicated. The species to be used, however, presented special difficulties. The irradiation of seeds seemed quite impractical because such effects as would be produced would probably be confined to sectors of the plant. Such limitation or isolation of the effects is to be expected as a result of the localization of X-rays to very small volumes which are sub-microscopic and to the relatively high state of development of the oat embryo. The consequence of such circumstances is that the sample taken for determination could be unique. If it included an affected region,

the remainder of the plant could be unaffected; and if it should include an unaffected area some other tiller could have included the desired deficiency. The male gamete seems to be the most useful nucleus to treat. It is already formed in mature oat pollen. The effect could not be lost before being incorporated in the zygote owing to pollen tube divisions, because this division, which is characteristic of many plants, does not occur in the cultivated cereals. The pollen grains cannot be treated because the flowers of oats are very delicate, and the seed set under artificial pollination is usually very low and always is unpredictable. Securing several hundred treated seeds from hand pollinations is too expensive in time and effort, if it is possible at all. The effect of X-ray treatment of the pollen would be to augment the difficulties already present. The only other nucleus which would not ordinarily produce sectorial effects is the egg nucleus. This can be treated easily with little or no reduction in seed set. Unfortunately, however, in oats, eggs cannot be treated profitably as individual units in the same manner that pollen can be treated. Such treatment would require that the flowers be emasculated. Successful large-scale emasculation and subsequent effective pollination of oats is still impossible. The only recourse under the present circumstances and state of knowledge is to irradiate whole flowers. The treatment will also affect the pollen, and the results obtained will represent a confounding of pollen treatments, egg treatments, certation and other unrecognized effects. Such treatment will not reduce the efficiency of the technique but may even augment it. It will, however, make difficult, if not impossible, the formulation of any hypothesis concerned with rates of effects or susceptibilities to radiation.

The way in which the radiation may act to produce monosomic plants is based on the assumption that the radiation produces a break in the two chromatids of a chromosome, with consequent formation of a dicentric chromosome which is involved in a breakage-fusion-breakage cycle during the early divisions of the cells of the embryo. Such chromosomes are lost, and the plant becomes monosomic for the affected chromosome.

The yield of 20 verified monosomes from 279 treated eggs would seem to justify a trial of the method on any species in which monosomes cannot be readily obtained. Such species are those which do not form asynaptic hybrids with related species or cannot be induced to form anomalous gametes by other means.

Although no quantitative data were secured on the nature of the chromosomes affected by the radiation, the observations did seem to indicate that most of the monosomes involved the smaller chromosomes of the genome. Whether this is correct cannot be known from the present sample which is not large enough to be considered adequate or representative. Since oats are certainly hexaploid they must contain enough duplication in the genome to permit the existence of deficiencies of the same character and magnitude as those known for wheat, a similar species cytologicaly (SEARS, 1944).

The nullisomes in oats were frequently partialy asynaptic to the extent that one or even two pairs of chromosomes failed to be bivalent at meiosis. Such lack of association may be seen in Figures 18 and 42 of nullisomes 210 and *m*. This, to some extent, confirms the observations of HUSKINS and HEARNE (1933) and would also seems to confirm the hypothesis of NISHIYAMA (1933) that the synapsis of meiotic chromosomes may be controlled by one gene. The deficieneies represented in these nullisomic plants presumably do not include deficiencies of the main gene essential for a wholly normal meiosis, i. e. the gene in chromosome C.

Meiosis in monosomic plants was characteristic of the phenomenon in wheat and tobacco. The univalent always occurred as a laggard at the first or second divisions, and appeared in the tetrads as one or two micronuclei (Figure 29). The division of the univalent seemed to occur in anaphase I, as may be seen in Figures 14, 25, and 26, and sometimes in anaphase II, as may be seen in Figure 15. This aspect of meiosis was not studied with respect to the different monosomics available.

The frequency of nullisomics was quite large in the progeny of some monosomic plants, assuming that the male and female behavior in sporogenesis was identical (NISHIYAMA, 1931), and that the composition of the quartets so far as it was possible to observe is of type C with 2(20), 2(20) + 2(1). In fact, from monosome *m* a high percentage of nullisomic plants may be expected, around 44 %, and such a high percentage of segregated nullisomes may be explained if the nullisomic pollen can compete successfully with normal pollen, and, as also could be expected, the female behavior is different from that on the male side. Studies of the megasporogenesis as done for other species (MORRISSON, 1953 and DAS, 1953) are quite desirable.

Though some cytological comparison of the univalents and a study of the meiotic behavior in the aberrant plants has been made and, so far as it was possible, identification of each one of the monosomics by comparative morphology of the nullisomic segregated, and of percentage in which they were segregated, has been tried, the work is now far from completed and will require several years of study and careful attention.

As mentioned before the season in which the plants are grown is very important, in spite of the normal control of the temperature and light available in a greenhouse. Three different periods were tried in starting the progenies, in the middle of the fall, late in the winter, and late in the spring.

In the first season there is a sharp difference between the development of the nullisomic plants and the monosomic and normal plants. Among the plants of this group and for some of the monosomes, it is possible to distinguish between normal and monosomic plants. In the second season it is practically impossible to find differences between the monosomes and plants with the normal set of chromosomes. In the third season the difference between the monosomic and nullisomic plants is most difficult to discover. At the beginning of the fall the new tillers of the monosomic plants grow normally and change completely the general morphology of the monosomic plants, if the temperature is lower, as is usual at this time.

Distinction among the different monosomes has been tentatively done by cytological observation but only monosome 39, Figure 30, was easily distinguished from the others, in the present series of observations.

The morphology of the nullisomic and sometimes the monosomic plant was the main tool used to classify the first ten monosomes, Figures 2, 3, 4, 5, 6. The existence of fertile nullisomic plants as shown in the last observations of some nullisomics, nullisomics 139 (Figure 11), 206, 214, 234, is a more important and decisive tool available for future work.

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RESUMO

Um tratamento com raios X de 300 unidades *r* pareceu ser eficiente na indução da condição monosómica da aveia cultivada, de 21 cromosomas, *Avena sativa* L. O tratamento foi aplicado às panículas, antes da antese, e por forma que os óvulos maduros e o pólen fossem tratados. Este tipo de tratamento tornou-se necessário dada a dificuldade das polinizações artificiais em quantidade compatível com as necessidades do trabalho.

A produção de monosómicos foi ligeiramente superior a 7 %. As frequências dos nulisómicos provenientes dos vários monosómicos variaram de 2 % a mais de 40 %, em amostras de, relativamente, pequenos números. Alguns dos nulisómicos produziram pólen e óvulos funcionais.

O método descrito parece ser aplicável a qualquer espécie que possa tolerar a deficiência de todo um cromosoma em qualquer dos gametas feminino ou masculino.

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PLATE I

Fig. 1 — normal disomic plant of *Avena sativa* L.
Fig. 2 — nullisome *b*
Fig. 3 — monosome *c*
Fig. 4 — nullisome *f*
Fig. 5 — monosome *f*
Fig. 6 — nullisome *m*

PLATE II

Fig. 7 — disome, monosome and nullisome 39
Fig. 8 — disome, monosome and nullisome 254
Fig. 9 — disome, monosome and nullisome *m*
Fig. 10 — nullisome and monosome *j*
Fig. 11 — disome, monosome and nullisome 139
Fig. 12 — monosome *b*, early telophase I
Fig. 13 — translocation, metaphase I
Fig. 14 — monosome *b*, early telophase I

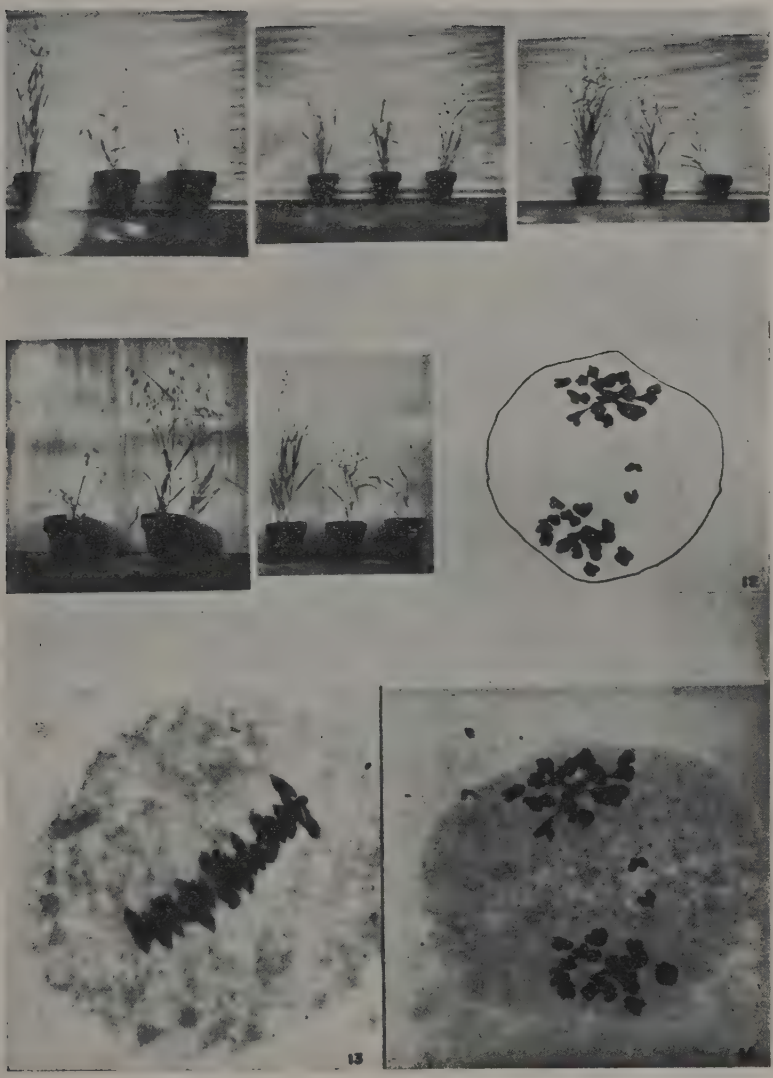

PLATE III

Fig. 15 — monosome 180, telophase II
Fig. 16 — monosome 240 and fragment, metaphase I
Fig. 17 — monosome 180, telophase II
Fig. 18 — nullisome 210 and centralized fragment, diakinesis
Fig. 19 — nullisome 210 and centralized fragment, diakinesis

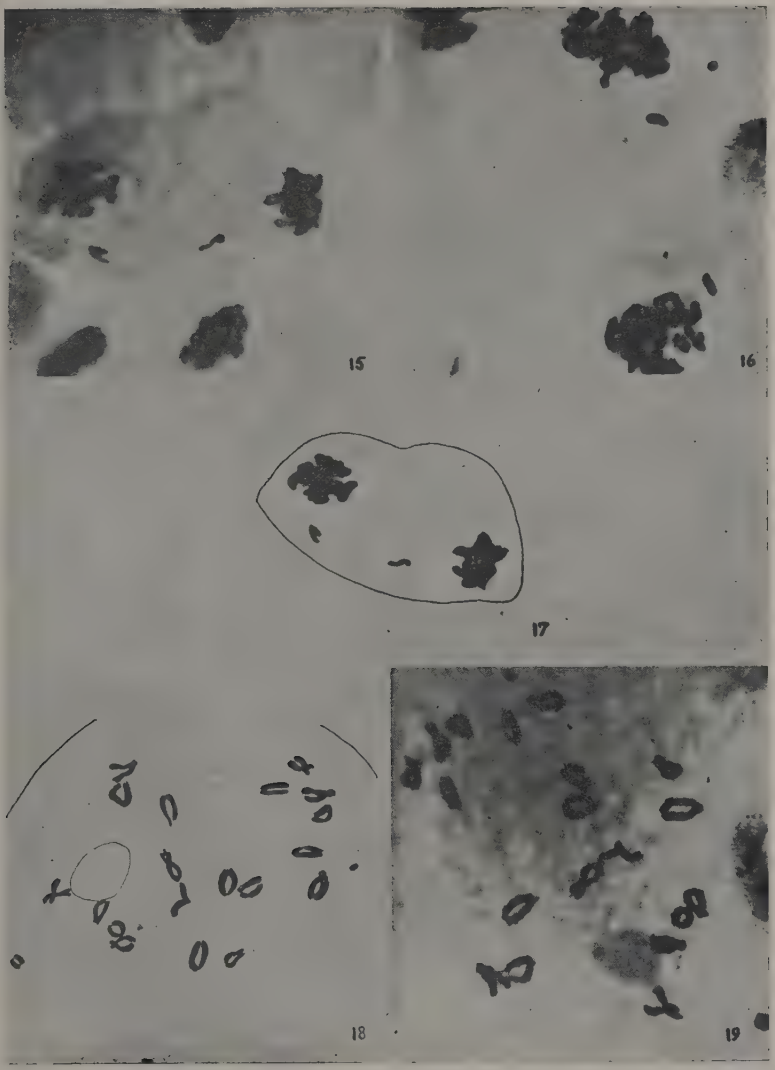
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PLATE IV

Fig. 20 — monosome 254, telophase I
Fig. 21 — monosome 254, diakinesis, 21 II + 1 I
Fig. 22 — monosome 254, diakinesis, 21 II + 1 I
Fig. 23 — monosome 254, diakinesis, 19 II + 1 I
Fig. 24 — monosome 254, diakinesis, 19 II + 1 I

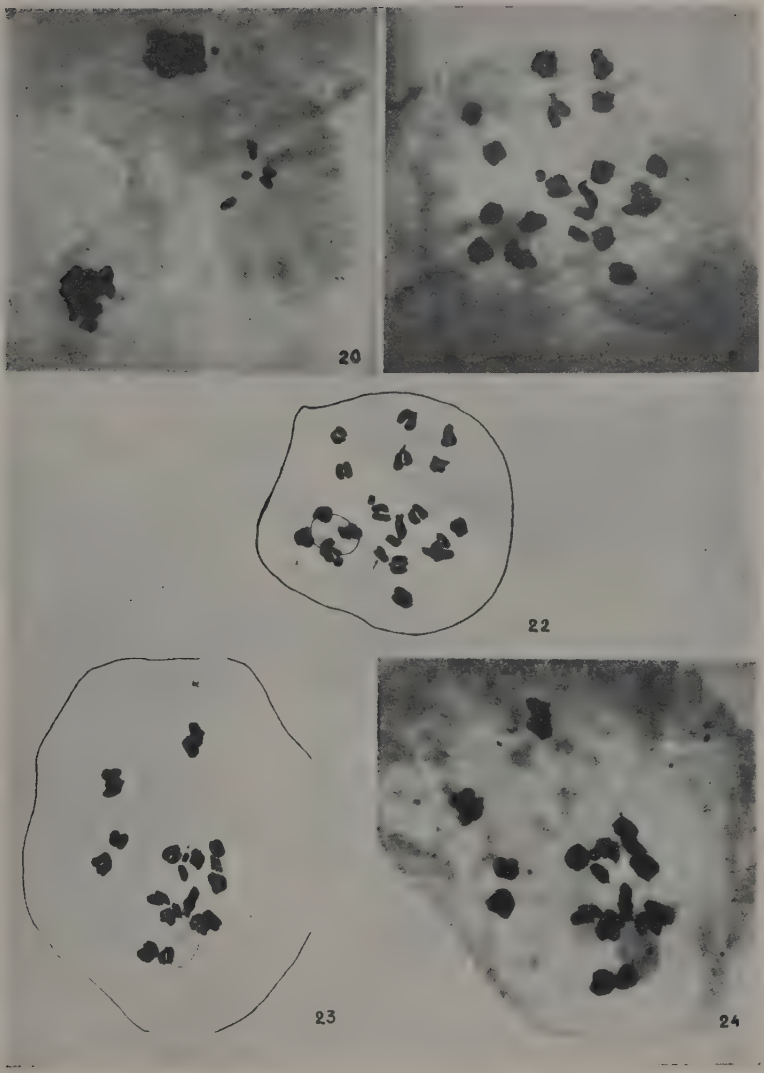
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PLATE V

Fig. 25 — monosome *b*, quartet stage
Fig. 26 — monosome 271, anaphase I
Fig. 27 — monosome *b*, quartet stage
Fig. 28 — monosome *h*, telophase I
Fig. 29 — monosome *b*, quartet stage

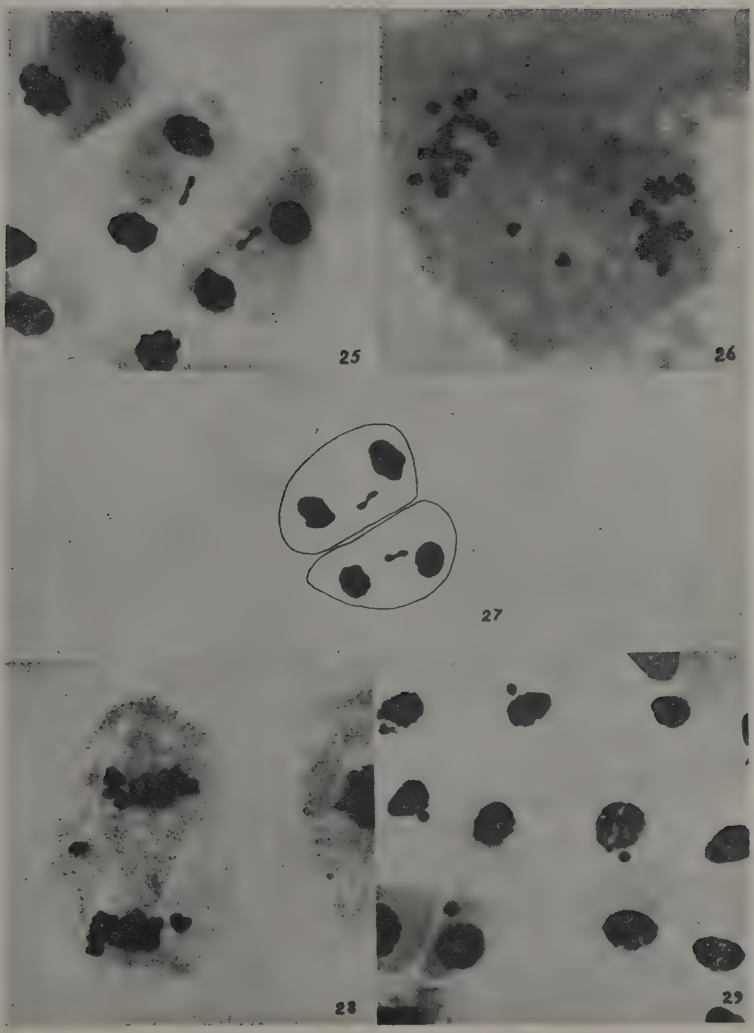
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PLATE VI

Fig. 30 — monosome 39, telophase II
Fig. 31 — monosome 39, telophase II
Fig. 32 — monosome 165, quartet stage
Fig. 33 — monosome 165, quartet stage
Fig. 34 — monosome *h*, metaphase I
Fig. 35 — monosome 226, late anaphase I

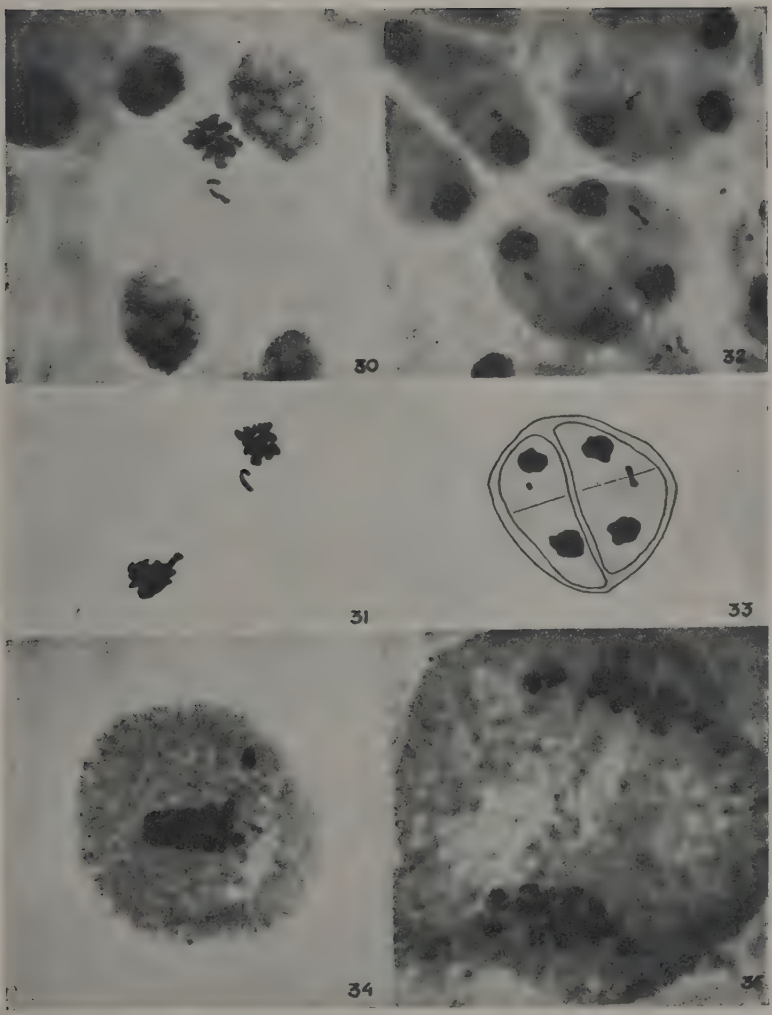
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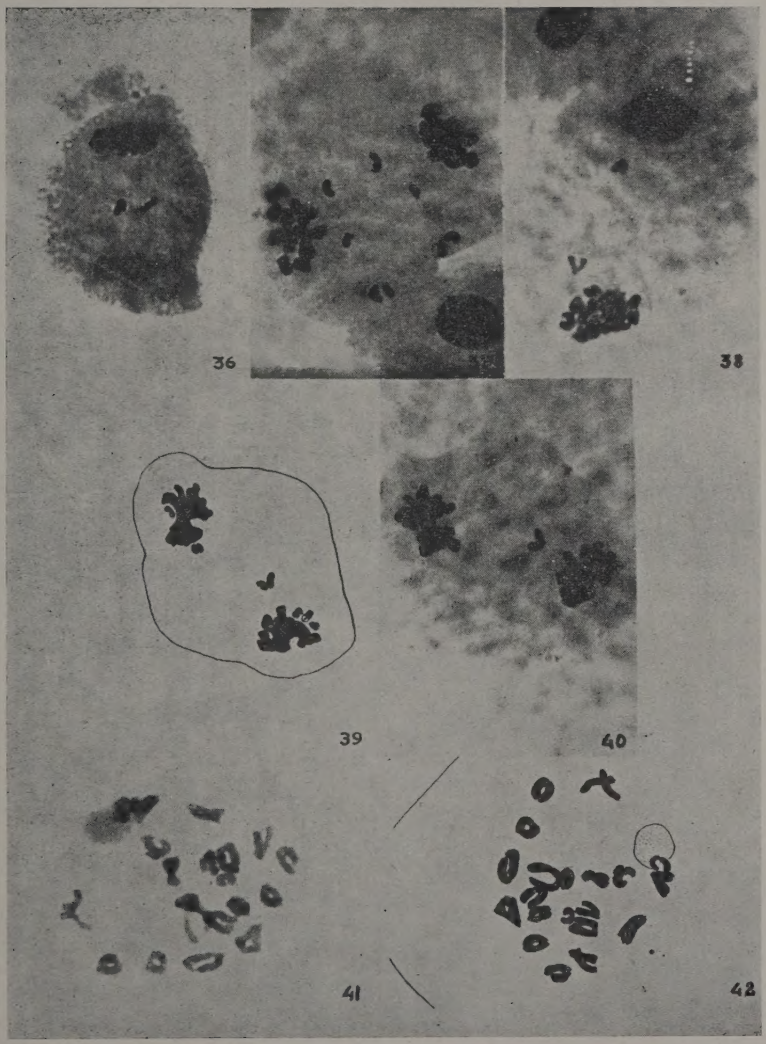
PLATE VII

Fig. 36 — monosome 206, telophase II
Fig. 37 — nullisome 139, anaphase I
Fig. 38 — monosome 139, telophase I
Fig. 39 — monosome *l*, telophase I
Fig. 40 — monosome *l*, telophase I
Fig. 41 — nullisome *m*, diakinesis
Fig. 42 — nullisome *m*, diakinesis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PUBLICAÇÃO DA
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TODA A CORRESPONDÊNCIA E PERMUTA REFERENTE À AGRONOMIA LUSITANA DEVE SER DIRIGIDA A:

POUR L'ÉCHANGE OU POUR LA CORRESPONDENCE CONCERNANT L'AGRONOMIA LUSITANA ON DOIT S'ADDRESSER À:

LETTERS AND EXCHANGE CONCERNING AGRONOMIA LUSITANA TO BE ADDRESSED TO:

ESTAÇÃO AGRONÓMICA NACIONAL
SACAVÉM
PORTUGAL